Neue Erkenntnisse über die Taxonomie, Systematik und Evolution von Süßwasserschnecken (Viviparidae und Truncatelloidea) aus China

Novel insights into the taxonomy, systematics and evolution of freshwater gastropods (Viviparidae and Truncatelloidea) from China

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To my parents

Taxonomic disclaimer

This publication is not deemed valid for taxonomic purposes (see Article 8.2. in the International Code of Zoological Nomenclature, 4th edition (1999).

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1 ABSTRACT

This thesis compiles a comprehensive set of case studies on the taxonomy, systematics and evolution of two most diverse groups of freshwater gastropods from China, family Viviparidae (Mollusca, Gastropoda, Architaenioglossa) and superfamily Truncatelloidea (Mollusca, Gastropoda, Littorinimorpha). These two groups represent some of the most ancient lineages of freshwater gastropods, which can be traced back to the Late Triassic (Truncatelloidea) and the Early Jurassic (Viviparidae). They both have worldwide distributions and play important roles in aquatic ecosystems. These two groups also have been studied for a long time because of their medical importance as disease vectors, commercial value as food resources, and the scientific interest in their biogeography and evolution.

China is the country harbouring the highest diversity of Viviparidae, and a vastly underestimated diversity of Truncatelloidea. However, scientific studies about these two groups from China are relatively scarce. Based on an integrative approach combining morphology, anatomy, molecular methods, ecology and biogeography, the present thesis provides a series of systematic revisions and the most complete list of valid species of Viviparidae from China so far. Within Viviparidae, two new genera, nine new extant species, one new fossil species, 11 new combiniations and one new synonym are proposed in the present thesis. The phylogenetic position of the new taxa within Viviparidae is discussed based on molecular phylogenetic trees. Two cases of non-adaptive radiation of Viviparidae are suggested from the plateau lakes of NW Yunnan Province and the Hainan Island of China.

With regard to Truncatelloidea, the present thesis provides the first fossil calibrated phylogeny based on five genes, covering most extant families of Truncatelloidea. One new family, one new genus and two new species all endemic to China are proposed herein. The sessile freshwater gastropod *Helicostoa* lost for more than a century is rediscovered and systematically studied for the first time. Besides its sessile life style in freshwater, the new species of *Helicostoa* represents one of the most remarkable cases of sexual dimorphism within molluscs: only females are sessile and develop a secondary aperture, while males are free-living and have a normal shell and aperture. The sessility of *Helicostoa* species is considered as an adaptation to the limestone habitat in large rivers. The extreme sexual dimorphism and secondary aperture of females are considered as further adaptations to solve the problems associated with mating and feeding coming with a sessile life history. The molecular, morphological and anatomic studies all confirm that *Helicostoa* should be assigned to Bithyniidae instead of a monotypic family Helicostoidae.

The fossil calibrated phylogeny of Truncatelloidea suggests that the unique freshwater microgastropod species from Fuxian Lake of China should be a new species of a new genus within a new family, Squamapicidae. fam. nov.. The dated phylogeny reveals that this new family of freshwater gastropods originated in the Tethys Ocean during the Late Cretaceous. In addition, this freshwater gastropod has a two-stage ornamented protoconch, which formerly has only been reported from marine or migratory gastropods with veliger larvae.

Overall, this thesis not only revises and describes many taxa of Viviparidae and Truncatelloidea from China, but also provides a more robust systematic frameworks for these two ancient snail groups. It also reveals several interesting cases of species radiations and evolutionary innovations, which contribute to a better understanding of their evolution.

ZUSAMMENFASSUNG

Diese Arbeit stellt eine umfassende Reihe von Fallstudien zur Taxonomie, Systematik und Evolution von zwei sehr unterschiedlichen Gruppen von Süßwasserschnecken aus China zusammen, der Familie Viviparidae (Mollusca, Gastropoda, Architaenioglossa) und der Überfamilie Truncatelloidea (Mollusca, Gastropoda, Littorinimorpha). Diese beiden Gruppen sind mit die ältesten Linien von Süßwasserschnecken, die bis in die späte Trias (Truncatelloidea) und den frühen Jura (Viviparidae) zurückverfolgt werden können. Beide sind weltweit verbreitet und spielen eine wichtige Rolle in aquatischen Ökosystemen. Diese beiden Gruppen werden auch seit langem wegen ihrer medizinischen Bedeutung als Krankheitsüberträger, ihres kommerziellen Wertes als Nahrungsquelle und wegen des wissenschaftlichen Interesses für ihre Biogeografie und Evolutionsbiologie.

China ist das Land mit der größten Vielfalt an Viviparidae und einer stark unterschätzten Vielfalt an Truncatelloidea. Wissenschaftliche Studien über diese beiden Gruppen aus China sind jedoch relativ rar. Auf der Grundlage eines integrativen Ansatzes, der Morphologie, Anatomie, molekulare Methoden, Ökologie und Biogeographie kombiniert, liefert die vorliegende Arbeit eine Reihe systematischer Revisionen und die bisher vollständigste Liste gültiger Arten der Viviparidae aus China. Innerhalb der Viviparidae werden in der vorliegenden Arbeit zwei neue Gattungen, neun neue rezente Arten, eine neue fossile Art, 11 neue Kombinationen und ein neues Synonym vorgeschlagen. Die phylogenetische Position der neuen Taxa innerhalb der Viviparidae wird anhand molekularphylogenetischer Bäume diskutiert. Zwei Fälle von nicht-adaptiver Radiation der Viviparidae werden aus den Plateauseen der NW-Provinz Yunnan und der Insel Hainan in China diskutiert.

In Bezug auf die Truncatelloidea liefert die vorliegende Arbeit die erste fossil kalibrierte Phylogenie auf der Grundlage von fünf Genen, die die meisten existierenden Familien der Truncatelloidea abdeckt. Eine neue Familie, eine neue Gattung und zwei neue Arten, die alle in China endemisch sind, werden hier beschrieben. Die seit mehr als einem Jahrhundert verschollene sessile Süßwasserschnecke *Helicostoa* ist wiederentdeckt und hier erstmals systematisch untersucht worden. Neben ihrer sessilen Lebensweise im Süßwasser stellt die neue *Helicostoa*-Art einen der bemerkenswertesten Fälle von Sexualdimorphismus bei Mollusken dar: nur die Weibchen sind sessil und entwickeln eine sekundäre Öffnung, während die Männchen freilebend sind und eine normale Schale und Öffnung haben. Die Sesshaftigkeit der Helicostoa-Arten wird als eine Anpassung an das Leben auf Kalkfelsen in großen Flüssen angesehen. Der extreme Sexualdimorphismus und die sekundäre Öffnung der Weibchen werden als weitere Anpassungen angesehen, um die mit einer sessilen Lebensweise verbundenen Probleme bei der Paarung und Ernährung zu lösen. Die molekularen, morphologischen und anatomischen Studien bestätigen, dass Helicostoa zu den Bithyniidae und nicht in eine monotypische Familie Helicostoidae gestellt werden sollte.

Die fossil kalibrierte Phylogenie der Truncatelloidea deutet darauf hin, dass die einzigartige Süßwasser-Mikrogastropodenart aus dem Fuxian-See in China eine neue Art einer neuen Gattung innerhalb einer neuen Familie, der Squamapicidae fam. nov., sein sollte. Die datierte Phylogenie zeigt, dass diese neue Familie von Süßwasserschnecken ihren Ursprung im Tethys-Ozean während der späten Kreidezeit hat. Darüber hinaus hat diese Süßwasserschnecke zweistufige, skulptierte Protokonche, die bisher nur von marinen oder migrierenden Schnecken mit Veliger-Larven bekannt waren.

Insgesamt werden in dieser Arbeit nicht nur viele Taxa der Viviparidae und Truncatelloidea aus China revidiert und beschrieben, sondern es wird auch ein robusterer systematischer Rahmen für diese beiden alten Schneckengruppen geschaffen. Sie zeigt auch mehrere interessante Fälle von Artenausbreitung und evolutionären Innovationen auf, die zu einem besseren Verständnis ihrer Evolution beitragen.

2 INTRODUCTION

2.1 Diversity and a brief history of discovery of freshwater gastropods from China

Gastropoda is one of the largest and most successful animal groups conquering the freshwater environment. Freshwater gastropods can be found worldwide currently except for the Antarctica (Lydeard & Cummings, 2019). They have adapted to a wide range of habitats, ranging from seasonal ponds to ancient lakes, mountain creeks to large rivers, and springs to subterranean water. There are 34 families, 535 genera and around 4370 species of gastropods restricted to freshwater (Lydeard & Cummings, 2019). Freshwater gastropods play an important role in aquatic ecosystems (Strong *et al*., 2008), have medical importance as disease vectors (Lydeard & Cummings, 2019), and are targeted for commercial harvesting in many places, especially Asia (Wen *et al*., 2018; Ng *et al*., 2020; Laishram, 2021).

China is one of the countries with the highest biodiversity globally. Among the 36 global biodiversity hotspots (https://www.conservation.org/priorities/biodiversity-hotspots), four hotspots are almost entirely ("Mountains of Southwest China") or partly ("Himalaya", "Mountains of Central Asia", "Indo-Burma") located in China. The various freshwater habitats in China harbour a high diversity of freshwater gastropods as well: Zhang *et al*. (1997) recorded 124 species from Yunnan Province; Shu *et al*. (2014) recorded 213 species, including 144 endemic species, from the Yangtze River Basin; Bai *et al*. (2022) recorded 318 species within 61 genera and 14 families from all over China. Yunnan Province is considered as the region in China with the highest biodiversity of freshwater gastropods (Zhang *et al*., 1997; Zhang *et al*., 2015). Meanwhile, the Yangtze River Basin is considered as the main hotspot of freshwater gastropods throughout China (Cai *et al*., 2018).

The earliest study of freshwater gastropods from China was based on specimens collected on Chusan Island (Zhoushan Island, Zhejiang Province), including a new species of Viviparidae, *Sinotaia quadrata* (Benson, 1842). Later, Reeve (1850) described a species of Lymnaeidae from Tibet. Adams (1866) described a species of Lymnaeidae from Taiwan Island. Nevil (1877), Neumayr (1883) and Mabille (1886) described several endemic species of Viviparidae from Yunnan. Pierre Marie Heude was the first and most important researcher who systematically collected and described species of non-marine molluscs throughout China, especially in the Yangtze River Basin (Heude, 1882-1890). He described almost 600 species, including at least 70 species of freshwater gastropods (Johnson, 1973). Dautzenberg & Fischer (1905) and Annadale (1924) studied and described more freshwater gastropods from Yunnan. Ping (1938) described a new species of Viviparidae and four new species of freshwater gastropod "*Melania*" from Anhui. Yen (1939) studied the specimens of freshwater gastropods from China kept in Senckenberg Museum, Frankfurt; he revised the recent species of Chinese Viviparidae for the first time and described several species (Yen, 1943).

Si Tchang is one of the pioneer Chinese researchers who conducted field works and studies of freshwater gastropods in China. He revised the genus *Margarya* of Viviparidae endemic to Yunnan and described one new species (Tchang & Tsi, 1949). Si Tchang and Yueying Liu organized and conducted a series of surveys on freshwater gastropods in many places of China, such as Tibet (Liu,

1963), the Yellow River Basin (Liu & Huang, 1964) and Dongting Lake (Tchang *et al*., 1965). Liu *et al*. (1979) wrote the first comprehensive monograph focusing on the freshwater molluscs of China. Since 1980s, the number of new freshwater gastropod species from China has increased dramatically, and most of them are species of Viviparidae and Truncatelloidea (Liu *et al*., 1980; Zhang *et al*., 1981; Liu *et al*., 1982; Davis *et al*., 1983; Liu *et al*., 1983a; Liu *et al*., 1983b; Davis *et al*., 1984; Davis *et al*., 1985; Liu *et al*., 1985; Davis *et al*., 1986a; Davis *et al*., 1986b; Davis *et al*., 1990a; Davis *et al*., 1990b; Liu *et al*., 1991; Chen *et al*., 2007).

During the last two decades, many comprehensive studies were conducted on Chinese freshwater gastropods. Liu *et al*. (2014) presented the first molecular phylogeny including many species of Pomatiopsidae from China. Based on morphology, anatomy and molecular studies, five new species of Pachychilidae (Köhler *et al*., 2010; Wang *et al*., 2018; Du & Yang, 2019; Liang *et al*., 2022), two new genera and four new species of Viviparidae (Du *et al*., 2011; He, 2013; Zhang *et al*., 2015; Lu, Fang & Du, 2016; Zhang, 2017), one new species of Planorbidae (Shu *et al*., 2013), one new genus, seven new species and four unnamed endemic evolutionary lineages of Lymnaeidae (Aksenova *et al*., 2018; Aksenova *et al*., 2019; Wiese *et al*., 2020; Vinarski *et al*., 2022), four new species of Semisulcospiridae (Du *et al*. 2019) and one new species of Pomatiopsidae (Shi *et al*., 2020) were described from China.

2.2 The systematics of Viviparidae - state of the art

Viviparidae is the only extant family within the superfamily Viviparoidea. It is a family of freshwater gastropods with medium diversity, which includes 125 to 150 valid described extant species within 29 extant genera (Strong *et al*., 2008; MolluscaBase, 2023). Viviparidae has a worldwide extant distribution except for South America and Antarctica (Strong *et al*., 2008).

Viviparidae has a rich fossil record (Prashad, 1928). The oldest fossil record from the late-Early Jurassic strata, *Viviparus sangongheensis* Zhu, 1994 †, was found in the Sangonghe Formation of Xinjiang, China (late Pliensbachian to early Aalenian; Li *et al*., 2023). From the Middle Jurassic strata, *Viviparus qiketaiensis* Zhu, 1994 † was found in the Qiketai Formation of Xinjiang, China, and *Bathonella scotica* (Tate, 1873) † was found in the Bathonian beds of North Oxfordshire, England, UK (Watson, 1950). Viviparidae had an almost worldwide distribution during the Late Jurassic, with fossil records from Europe (Stelbrink *et al*. 2020), Asia (Pan, 1977), North America (Chure *et al*. 2006), Australia (Frese & Ponder, 2021) and possibly South America (Perea *et al*. 2009). By the early Cretaceous, the distribtion of Viviparidae extended to Africa (van Damme *et al*. 2015) and conclusively South America (Simone & Mezzalira 1994). The worldwide distribution of Viviparidae since the Jurassic suggests an origin in Gondwana (Frese & Ponder, 2021).

Viviparidae is characterized by the right cephalic tentacle modified into a penis in males and the ovoviviparous reproduction with the distal pallial oviduct modified into a brood pouch in females (Strong, 2003; Van Bocxlaer & Strong, 2016). There are three subfamilies within Viviparidae: Bellamyinae, Viviparinae, and Lioplacinae. The monophyly of family Viviparidae and its three subfamilies is strongly supported by both anatomic studies (Vail, 1977; Strong, 2003; Van Bocxlaer *et*

al., 2018;) and recent molecular studies (Hirano *et al*., 2019a; Stelbrink *et al*., 2020). The members of the three subfamilies can be easily differentiated based on the distinct structure of the male reproductive system (Rohrbach 1937; Vail, 1977). Bellamyinae has been recorded from Asia, Australia and Africa (Stelbrink *et al*., 2020); Viviparinae has been recorded from Europe, North America and East Asia (Stelbrink *et al*., 2020; Van Bocxlaer *et al*., 2018; Hirano *et al*., 2019a); Lioplacinae currently is only known from North America, but there is an extremely rich fossil record in the Jurassic to Cretaceous strata of China (species of *Lioplacodes* Meek, 1864 †: Yü, 1974; Pan, 1977; Yü & Pan, 1980; Yu, 1982; Guo *et al*., 1982; Pan, 1982; Yü, 1982; Yü, 1987; Gu & Wang, 1989; Xiao *et al*., 1994), South America (species of *Lioplacodes*: Parodiz, 1969 †) and Europe (species of *Lioplacodes*: Yen, 1966; species of *Scalipaludina* Starobogatov, 1986 †: Matheron, 1832; Starobogatov, 1986).

Viviparidae have recently received increased attention by researchers studying their systematics, evolutionary history and biogeography. Sengupta *et al*. (2009) presented the first molecular phylogeny of Viviparidae including all subfamilies and 12 genera, revealing three geographical lake-clades of the species from the African Great Lakes. Schultheiß *et al*. (2014) revealed the role of Congo River Basin in shaping the distribution pattern of the Viviparidae species from the African Great Lakes. Du *et al*. (2013) firstly confirmed that the viviparid genus *Margarya* endemic to Yunnan, China is polyphyletic, and Zhang *et al*. (2015) elevated and erected two new viviparid genera from *Margarya* based on morphology, anatomy, distribution and molecular phylogeny. Hirano *et al*. (2015) presented the first phylogeny including all viviparid species from Japan. Based on anatomy and a multi-locus molecular phylogeny, van Bocxlaer *et al*. (2018) confirmed that the viviparid genus *Rivularia* Heude, 1890 endemic to China belongs to subfamily Viviparinae. The widespread species *Heterogen japonica* (Martens, 1861) and *Heterogen longispira* (Smith, 1886) endemic to Biwa Lake can hardly be differentiated either in a multi-locus phylogeny or using genome-wide SNPs (Hirano *et al*. 2019a). Stelbrink *et al*. (2020) presented the most complete time-calibrated molecular phylogeny of Viviparidae, including 24 of 28 genera, revealing the most relatable systematic relationship between genera to date.

China is the country with the highest diversity of Viviparidae globally, with at least 45 species belonging to two subfamily and 13 genera, and four genera endemic to China (Liu *et al*. 1995; Zhang *et al*., 2015; van Bocxlaer *et al*., 2018). However, the latest large-scale revision of Chinese Viviparidae by Liu *et al*. (1995) was only based on shell morphology. The studies of Zhang *et al*. (2015) and Wiese *et al*. (2022) have indicated that the species delimitation of Viviparidae solely based on shell morphology is not reliable enough due to the high inter- and intraspecific variation and convergent evolution of viviparid shells.

2.3 The systematics of Truncatelloidea - state of the art

Truncatelloidea Gray, 1840 is one of the largest and most diverse superfamilies within Gastropoda or even all Mollusca, including 35 families (33 extant), 715 genera, and at least 6739 accepted species (MolluscaBase., 2021). Members of this superfamily are distributed across all oceans and continents,

with the exception of Antarctica. They exhibit an extremely broad range of adaptions to various habitats – marine (including the bathyal zone), brackish, freshwater and terrestrial; the most diverse group "hydrobioid" is found in freshwater (MolluscaBase., 2021; Lima *et al*., 2016; Rubio, *et al*., 2016; Arango & Kintz, 2021). Truncatelloidea has a rich fossil record (MolluscaBase., 2021). The oldest fossil of this superfamily, *Triasamnicola pilsbryi* Yen & Reeside, 1946, is a freshwater gastropod from the Late Triassic strata of the Chinle Formation, USA (Kirby, 1993).

The species comprising Truncatelloidea are all minute to small in size, typically ranging from 0.7 mm (Spirostyliferinidae Layton, Middelfart, Tatarnic & N. G. Wilson, 2019) to 15 mm (Bithyniidae Gray, 1857), posing challenges to taxonomic studies in the past. Most species of Truncatelloidea were assigned to Rissooidea Gray, 1847. However, with the help of a molecular phylogeny based on 16S and 28S markers (Criscione & Ponder, 2013), the superfamily Truncatelloidea was erected as a distinct monophyletic lineage from Rissooidea. Several subsequent molecular studies have confirmed the validity of Truncatelloidea (Takano & Kano, 2014; Golding, 2014a; Golding, 2014b). A molecular phylogeny revealed that the marine snail family Caledoniellidae Rosewater, 1969 should be assigned to Truncatelloidea (Goto *et al*., 2021). Two hydrobioid families, Baicaliidae Fischer, 1885 and Erhaiidae Davis & Kuo, 1985, have been considered as valid based on morphological and molecular evidence, and a fossil family †Palaeobaicaliidae Sitnikova & Vinarski, 2023 from the Late Cretaceous has been proposed (Sitnikova *et al*., 2023). A monotypic family, Litthabitellidae Falniowski & Hofman, 2023, has been erected for the hydrobioid genus *Litthabitella* Boeters, 1970 (Falniowski *et al*., 2023). Wilke *et al*. (2023) demonstrated by using a museomics approach that the freshwater truncatelloidean family Helicostoidae Pruvot-Fol, 1937 with a sessile habit should only be a subfamily-level group of Bithyniidae Gray, 1857.

The "hydrobioids" are the most diverse group within Truncatelloidea. This name formerly was used on almost all small freshwater gastropods within Rissooidea, especially for species of the family Hydrobiidae. Hydrobioids comprise more than 1000 described species and more than 400 recent and fossil genera, and the group has been estimated to contain a total of around 4000 species (Hershler & Ponder, 1998; Strong *et al*., 2008). Hershler & Ponder (1998) provided the most comprehensive review of morphological characters of hydrobioid snails to date. Davis *et al*. (1998) presented the first molecular tree of hydrobioids. This study revealed that the family Hydrobiidae was polyphyletic, and several subfamilies of Hydrobiidae should be elevated to full family level. A series of subsequent studies by Thomas Wilke's group (Wilke *et al*., 2000; Wilke *et al*., 2001; Wilke *et al*., 2013) further confirmed that many genera of hydrobioids should be elevated to family level.

Layton *et al*. (2019) presented the most complete phylogeny of Truncatelloidea at family level based on a combined data sets of five genes. Their study included 20 of 33 extant families, and a new monotypic family was erected for the marine truncatelloidea genus *Spirostyliferina* Bandel, 2006. In this phylogeny, Truncatelloidea was divided into two highly supported main lineages. One lineage included most marine families and the other one included most freshwater families, viz. the "hydrobioid" lineage. Their works also revealed the close relationship of several families, such as the highly supported clade of Assimineidae, Falsicingulidae and Pomatiopsidae. However, the

phylogenetic position of most families is still uncertain in this tree due to the low support values of most nodes. Sitnikova *et al*. (2023) used the divergence rate of COI for calibrating the first molecular clock study including multiple families of hydrobioids. However, there is no fossil calibrated molecular clock study covering most families of Truncatelloidea so far.

2.4 Aims and chapter overview of the thesis

The general major aim of the thesis is to review the systematics of Viviparidae and Truncatelloidea from China. In detail, the present thesis pursues the following three objectives:

1) To answer the question how many species and genera of Viviapriade are currently known from China, and to discover and describe new taxa, based on comprehensive studies of historic and fresh material combining data from morphology, anatomy, mtDNA (COI barcoding marker), ecology and distribution.
2) To present a fossil calibrated phylogeny of Truncatelloidea at family level based on mitochondrial and nuclear genes, with a focus on the freshwater truncatelloidean groups endemic to China, including the former monospecific family Helicostoidae and a new monospecific family endemic to an ancient lake of China.
3) To discuss the evolution of Viviparidae and Truncatelloidea, especially the radiation of Viviparidae in insular habitats (lakes and island), and the evolutionary innovation of two Chinese endemic truncatelloidean groups ("Helicostoidae" and the new family).

This thesis compiles a series of original studies focusing on Viviparidae and Truncatelloidea, the two most diverse freshwater gastropod groups from China. Prior to these studies, a very limited number of systematic studies of these two groups from China with modern research methods had been conducted. In these studies, one new family, three new genera, 12 new species and 12 new combinations have been proposed.

Chapter 1 (Zhang & von Rintelen, 2021) revises the characters of operculum of Viviparidae systematically for the first time, confirming the high taxonomic value of the operculum for genus and species identification. It provides an important methodological basis for subsequent morphological studies of Viviparidae.

Chapter 2 (Zhang *et al*., 2023) proposes a new viviparid genus *Dalipaludina* with four known species based on morphology and genetic analysis. The four species of this genus show a strictly allopatric distribution. This genus is endemic to the shallow lentic habitats of high altitude regions of Yunnan, China.

Chapter 3 (Zhang & Wang, 2023) reports an extinct freshwater mollusc fauna from Quaternary lacustrine deposits of a group of vanished lakes in Yunnan, China. This fauna has many species of Viviparidae and Truncatelloidea, and a new fossil species of viviparid *Tchangmargarya* was described.

Chapter 4 (Zhang *et al*., manuscript) proposes a new viviparid genus *Bakyietaia* with seven new combinations and nine new species, based on a comprehensive study of their morphology, genetic relationship and distribution. This genus contains an insular radiation on Hainan Island. The marine

regressions and transgressions in the South China Sea since the Pleistocene probably play an important role in the formation of the current distribution of this genus in South China and Mainland Southeast Asia.

Chapter 5 (Zhang *et al*., 2024) report the rediscovery of the enigmatic truncatelloidean genus *Helicostoa* with a unique sessile habit lost for more than a century. A new species of *Helicostoa* was described based on a comprehensive study of its morphology and molecular phylogenetics. The study revealed the remarkable sexual dimorphism within *Helicostoa*: only the adult female is sessile on the rock, and a secondary aperture is developed on the adult female's body whorl; the adult male is vagile and has one normal aperture.

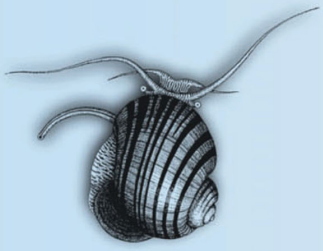
Chapter 6 (Zhang *et al*., manuscript) presented the first fossil calibrated phylogeny of Truncatelloidea at family level based on five mitochondrial and nuclear genes. A new freshwater truncatelloidean family Squamapicidae is described based on the phylogeny and its unique morphology. This family contains only one new species of a new genus endemic to Fuxian Lake of Yunnan, China. This freshwater gastropod has a two-stage ornamented protoconch, which formerly is only reported from marine or migratory gastropods with veliger larvae. The fossil calibrated phylogeny suggests a Cretaceous Tethys origin of this new family.

These publications and manuscripts will be presented in the following section.

3 RESULTS – PUBLICATIONS AND MANUSCRIPTS INCLUDED IN THIS THESIS

Chapter 1. Zhang LJ & von Rintelen T (2021). The neglected operculum: a revision of the opercular characters in river snails (Caenogastropoda: Viviparidae). *Journal of Molluscan Studies* **87**: eyab008.

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The neglected operculum: a revision of the opercular characters in river snails (Caenogastropoda: Viviparidae)

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ABSTRACT

The morphological identification of many groups of gastropods is mostly based on shell morphology and genital structures. The value of opercular characters as taxonomic characters has so far been barely studied and evaluated. Here, the opercular characters of river snails (Viviparidae) have been revised systematically for the first time. Based on a morphological study of the opercula of 38 species of 25 viviparid genera, 16 newly defined opercular characters are shown to be useful for genus and species identification. A detailed diagnosis and description of opercular characters for each genus/species is given. One new generic assignment of a viviparid species is proposed based on this study, namely *Cipangopaludina nagaensis* n. comb.

INTRODUCTION

The operculum is a disc-shaped structure attached to the dorsal surface of the foot in almost all 'prosobranch' and heterobranch gastropods (Checa & Jimenez-Jimenez, 1998). Opercula have been used in both extant and fossil species and genus identification for a long time (e.g. Sarasin & Sarasin, 1898; Walker, Dodge & Williamson, 1916; Yochelson & Linsley, 1972). Checa & Jimenez-Jimenez (1998) comprehensively studied operculum function, formation and types and discussed the systematic and evolutionary significance of the operculum.

However, systematic and comprehensive studies of the operculum are scarce compared to the numerous morphological studies of gastropods focusing almost entirely on the shell and genital structures. Simone (2004) included operculum comparisons of some representative species of Ampullariidae, Viviparidae, Neocyclotidae and Annulariidae in a phylogenetic study of Caenogastropoda. In lands snails, Wilmsmeier & Neubert (2012) discovered microcanals within the operculum of family Pomatiidae and confirmed the taxonomic and systematic value of opercular characters for this group; Foon & Liew (2017) used operculum characters in a taxonomic review of *Alycaeus* (Alycaeidae) and Cole (2019) employed diagnostic characters of the operculum in a key of *Chondrocyclus s. l.* (Cyclophoridae). Checa & Jimenez-Jimenez (1998) considered that all species of Viviparidae have a concentric operculum with a subcentral nucleus. However, Walker *et al.* (1916) had already pointed out that the operculum of the viviparid genus *Lioplax* has a paucispiral nucleus and concentric margin. A comprehensive study of three endemic genera of river snails (Viviparidae: *Margarya*, *Anularya* and *Tchangmargarya*) from Yunnan, China (Zhang *et al.*, 2015) revealed that opercular characters can be used to distinguish these three genera.

River snails (Viviparidae Gray, 1847) are an ancient, almost globally distributed freshwater snail family that originated in the Jurassic (Stelbrink *et al.*, 2020). Thus far, 28 extant genera and *c.* 125–150 species of this family have been considered valid (Strong *et al.*, 2008; Stelbrink *et al.*, 2020). Several recent studies of Viviparidae combining molecular and morphological data revealed conflicts between shell-based taxonomy and genetically based interspecific relationships (Sengupta *et al.*, 2009; Hirano, Saito & Chiba, 2015; Zhang *et al.*, 2015; Wang *et al.*, 2017; Hirano *et al.*, 2019a, b; Stelbrink *et al.*, 2020). Shell characters have been shown to be of limited use in distinguishing some genera, such as *Margarya*, *Anularya* and *Tchangmargarya* (Du *et al.*, 2013; Zhang *et al.*, 2015), or *Cipangopaludina* (*C. japonica*) and *Heterogen* (*H. longispira*) (Hirano *et al.*, 2015, 2019a). The most complete genus-level molecular phylogeny of Viviparidae produced to date (Stelbrink *et al.*, 2020) indicates that a large-scale revision of viviparid genera is due, which will require a rigorous re-assessment of their morphology and anatomy.

Here, the operculum of Viviparidae has been studied systematically for the first time in 25 genera and 38 species to investigate whether opercular characters are useful for differentiating viviparid taxa at the species and/or genus level.

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MATERIAL AND METHODS

Material and measurements

For each viviparid species studied ($n = 38$), from 1 to 11 opercula (119 in total) were examined (Table 1). Most of the studied material is deposited in the collection of the Museum für Naturkunde, Berlin (ZMB) and referred to by accession number. A few studied samples are in the private collections of Jiang Wang (WJ), Xiao-Dong Wang (WXD) and Le-Jia Zhang (ZLJ).

The opercula of ethanol-preserved specimens were detached from the foot with tweezers and cleaned with water to remove all remaining tissue. The opercula were photographed using a stereomicroscope (Leica MDG34) and a Leica Summilux-H1 1.6/27 ASPH camera. Measurements (length and areas) of different parts

Table 1. Species of Viviparidae examined.

Species	Museum acc. no.	Locality	No. of specimens examined
Campeloma decisum (Say, 1817) (Figs 2B, 3B, 6B)	ZMB Moll. 250824	"Indiana" (Indiana, USA)	1
	ZMB Moll. 122715	"U.S. Amer." (USA)	2
Lioplax subcarinata (Say, 1817) (Figs 2A, 3A, 6A)	ZMB Moll. 250815	"Indiana" (Indiana, USA)	6
Amuropaludina praerosa (Gerstfeldt, 1859) (Figs 2H, 3H, 6H)	ZMB Moll. 250816	"Amur Fluss" (Amur River)	2
	ZMB Moll. 260002	Amur River	1
Callinina georgiana (Lea, 1834) (Figs 2C, D, 3C, D, 6C, D)	ZMB Moll. 250827	"Florida, Black Creek" (Black Creek, Florida, USA)	3
	ZMB Moll. 250829	"Indiana" (Indiana, USA)	5
	ZMB Moll. 250825	"Florida" (Florida, USA)	3
Callinina intertexta (Say, 1829) (Figs 2E, 3E, 6E)	ZMB Moll. 250826	"Mississippi" (Mississippi, USA)	1
Rivularia auriculata (Martens, 1875) (Figs 2G, 3G, 6G)	ZMB Moll. 31048 (paratypes)	"Siangkiang Fluss, Hunan" (Xiang River, Hunan, China)	2
Tulotoma magnifica (Conrad, 1834) (Figs 2F, 3F, 6F)	ZMB Moll. 250817	"Alabama" (Alabama, USA)	3
Viviparus viviparus (Linnaeus, 1758) (Figs 2I, 3I, 6I)	ZMB Moll. 63883	"Waukawysk" (Waukawysk, Belarus)	5
Viviparus contectus Millet, 1813 (Figs 2J, K, 3J, K, 6J, K)	ZMB Moll. 76634	–	3
	ZMB Moll. 250830	"Griechenland" (Greece)	1
Angulyagra cf. *polyzonata* (Frauenfeld, 1862) (Figs 4T, 5T, 7T)	ZMB Moll. 250844	Nanning, Guangxi, China	2
Anularya mansuyi (Dautzenberg & Fischer, 1905) (Figs 4D, 5D, 7D)	ZMB Moll. 193465	Qilu Lake, Yunnan, China	2
	WJ	Xingyun Lake, Yunnan, China	1
	ZLJ	Xingyun Lake, Yunnan, China	1
Anularya bicostata (Tchang & Tsi, 1949) (Figs 4E, 5E, 7E)	WJ	Fuxian Lake, Yunnan, China	1
	WXD	Fuxian Lake, Yunnan, China	1
	ZLJ	Fuxian Lake, Yunnan, China	1
Bellamya phthinotropis (Martens, 1892) (Figs 4H, 5H, 7H)	ZMB Moll. 122821 (syntype; labelled "*Vivipara constricta* var. *phthinotropis* von Martens, 1892")	"Sirwa" (Sirua Island, Lake Victoria, southern Tanzania)	2
Bellamya trochlearis Martens, 1892 (Figs 4I, 5I, 7I)	ZMB Moll. 250839	"Ukerewe" (Ukerewe Island, Lake Victoria)	5
Celetaia persculpta (Sarasin & Sarasin, 1898) (Figs 4A, 5A, 7A)	ZMB Moll. 50760 (syntype)	"Poso See, C. Celebes" Lake Poso, Central Sulawesi, Indonesia	1
	ZMB Moll. 191078	South of Cape Panti, east shore of Lake Poso, Central Sulawesi, Indonesia	4
Cipangopaludina chinensis (Gray, 1834) (Figs 2Q, 3Q, 6Q)	ZMB Moll. 250832	"Ulsan, Korea" (Ulsan, Korea)	4
Cipangopaludina nagaensis n. comb. (Preston, 1914) (Figs 2O, 3O, 6O)	ZMB Moll. 62192 (syntype)	"Naga Hügel, Indien" (Naga Hills, India)	1
Cipangopaludina crassibuca (Sarasin & Sarasin, 1898) (Figs 2P, 3P, 6P)	ZMB Moll. 50779 (syntype)	"Poso See, C. Celebes" (Lake Poso, Central Sulawesi, Indonesia)	1
Filopaludina martensi (Frauenfeld, 1864) (Figs 4M, 5M, 7M)	ZMB Moll. 250840	"Siam" (Thailand)	5

Continued

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Table 1. Continued

Species	Museum acc. no.	Locality	No. of specimens examined
Filopaludina bengalensis (Lamarck, 1822) (Figs 4N, 5N, 7N)	ZMB Moll. 250841	–	2
Filopaludina gigantea (von dem Busch, 1843) (Figs 4O, 5O, 7O)	ZMB Moll. 250842	"Bengal"	1
Filopaludina luzonica (Kobelt, 1909) (Figs 4L, 5L, 7L)	ZMB Moll. 6835	"Luzon" (Luzon Island, Philippines)	6
Heterogen longispira (E. A. Smith, 1886) (Figs 2S, 3S, 6S)	ZMB Moll. 192022	Biwa Lake, Japan	1
	ZMB Moll. 38491	"Japan"	5
Heterogen japonica (Martens, 1861) (Figs 2T, 3T, 6T)	ZMB Moll. 192092	"Japan"	1
	ZMB Moll. 28126	–	3
	ZMB Moll. 6844	"Japan"	5
Idiopoma umbilicata (Lea, 1856) (Figs 4J, 5J, 7J)	ZMB Moll. 52112 (syntype; labelled "*Vivipara basicarinata* Kobelt, 1908")	"Phuc son, Annam" (Phúc Sơn, Nghệ An, Vietnam)	2
Idiopoma dissimilis (Müller, 1774) (Figs 4K, 5K, 7K)	ZMB Moll. 49607	"Lashio" (Lashio, Myanmar)	2
Margarya melanioides (Dautzenberg & Fischer, 1905) (Figs 2R, 3R, 6R)	ZMB Moll. 193463	Dianchi Lake, Yunnan, China	2
	WJ	Guanyinshan, Dianchi Lake, Yunnan, China	1
	ZLJ	Jianhu Lake, Yunnan, China	1
Mekongia jullieni (Deshayes, 1876) (Figs 2L, 3L, 6L)	ZMB Moll. 250831	"Indochina"	1
Neothauma tanganyicense E. A. Smith, 1880 (Figs 4G, 5G, 7G)	ZMB Moll. 250837	–	1
Notopala ampullaroides (Reeve, 1863) (Figs 4S, 5S, 7S)	ZMB Moll. 106691	Lake Kununurra at pump station, Kimberley region, Australia	2
Sinotaia angularis (Müller, 1774) (Figs 4C, 5C, 7C)	ZMB Moll. 250834	"Nord Fluss, Canton" (Guangzhou, Guangdong, China)	2
Taia naticoides (Kobelt, 1909) (Figs 4Q, 5Q, 7Q)	ZMB Moll. 49604	"Shan Staaten" (Shan State, Myanmar)	5
Taia shanensis (Kobelt, 1909) (Figs 4R, 5R, 7R)	ZMB Moll. 49604	"Shan Staaten" (Shan State, Myanmar)	1
Tchangmargarya yangtsunghaiensis (Tchang & Tsi, 1949) (Figs 2N, 3N, 6M)	WJ	Yangzonghai Lake, Yunnan, China	1
	WXD	Yangzonghai Lake, Yunnan, China	1
	ZLJ	Yangzonghai Lake, Yunnan, China	1
Tchangmargarya multilabiata (Zhang & Chen, 2015) (Figs 2M, 3M, 6N)	WJ	Changhu Lake, Yunnan, China	1
	WXD	Changhu Lake, Yunnan, China	1
	ZLJ	Changhu Lake, Yunnan, China	1
Torotaia mainitensis (Bartsch, 1907) (Figs 4F, 5F, 7F)	ZMB Moll. 230165	West shore of Lake Mainit, Agusan del Norte, Mindanao, Philippines	3
Trochotaia trochoides (Martens, 1860) (Figs 4P, 5P, 7P)	ZMB Moll. 250843	"Siam" (Thailand)	2
Ussuripaludina ussuriensis (Gerstfeldt, 1859) (Figs 4B, 5B, 7B)	ZMB Moll. 250835	"Ussuri" (Ussuri River)	3

of each operculum were made on the photos using ImageJ 1.8.0 (Rueden *et al.*, 2017).

Definition of terms

We use a number of terms to describe the morphology of the viviparid operculum. These are defined as follows:

(1) *Interior surface* (Fig. 1A): the opercular surface attaching the opercular disc to the foot.
(2) *Exterior surface* (Fig. 1B): the opercular surface opposite to the interior surface.
(3) *Inner margin*: the opercular margin closest to the columella of the shell when the operculum is retracted into the shell aperture.
(4) *Outer margin*: the opercular margin closest to the outer lip of the shell when the operculum is retracted into the shell aperture.
(5) *Nucleus* (Fig. 1B: e): the earliest part of the operculum during development; always visible from the exterior surface.

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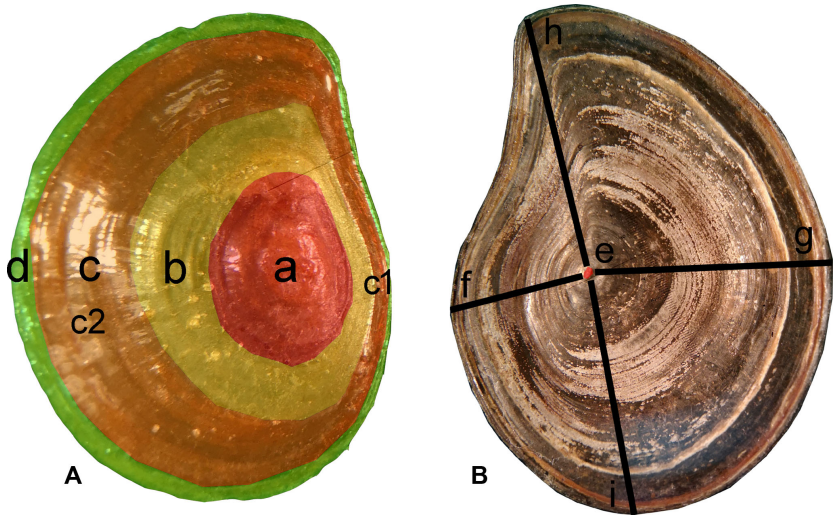


Figure 1. The viviparid operculum, showing the subregions and measured distances used in the study. **A.** Interior surface. **B.** Exterior surface. Abbreviations used: a, nuclear region; b, scar region; c, enamel region; c1, inner enamel region; c2, outer enamel region; d, marginal region; a + b, inner opercular region; c + d, outer opercular region; e, opercular nucleus; f, inner margin convex; g, outer margin convex; h, opercular apex; i, opercular base; line ef, inner-nucleus distance; line eg, outer-nucleus distance; line eh, apex–nucleus distance; line ei, base–nucleus distance.

(6) *Inner margin convex* (Fig. 1B: f): the most protruding point on the inner margin.
(7) *Outer margin convex* (Fig. 1B: g): the most protruding point on the outer margin.
(8) *Opercular apex* (Fig. 1B: h): the part of operculum closest to the suture of the shell when the operculum is retracted into the shell aperture.
(9) *Opercular base* (Fig. 1B: i): the part of operculum closest to the base of the aperture when the operculum is retracted into the shell aperture.
(10) *Inner opercular region* (Fig. 1A: a + b): the region on the interior surface directly attached to the opercular disc of the foot.
(11) *Outer opercular region* (Fig. 1A: c + d): the region on the interior surface not directly attached to the opercular disc of the foot.
(12) *Nuclear region* (Fig. 1A: a): the region of the inner opercular region, surrounding the nucleus and usually distinguishable from the remaining part of inner opercular region.
(13) *Scar region* (Fig. 1A: b): the remaining part of inner opercular region surrounding the nuclear region, sometimes not distinguishable.
(14) *Enamel region* (Fig. 1A: c): the region within the outer opercular region, always covered with a smooth and shiny layer, here called the enamel layer.
(15) *Inner enamel region* (Fig. 1A: c1): the part of enamel region close to the inner margin.
(16) *Outer enamel region* (Fig. 1A: c2): the part of enamel region close to the outer margin.
(17) *Marginal region* (Fig. 1A: d): the region of the outer opercular region, on the margin of the operculum, sometimes not differentiated from the enamel region.

Distribution of opercular characters among viviparids

Based on the investigation of 119 opercula from 38 species, a character matrix for the 25 viviparid genera studied was coded for 16 distinct binary characters (Table 2). The characters and character states are as follows:

(1) Growth striae: 0, concentric (e.g. Fig. 2B); 1, paucispiral nucleus and concentric margin (e.g. Fig. 2A).
(2) Position of nucleus on the plane of the operculum (quantified as inner-nucleus distance:outer-nucleus distance): 0, subcentral (>1:5 or <1:1) (e.g. Fig. 2B); 1, central (1:1) (e.g. Fig. 2A).
(3) Ratio between distance from apex to nucleus and distance from base to nucleus: 0, 1 (e.g. Fig. 2C); 1, >1 (e.g. Fig. 2B).
(4) Shape: 0, inverted-comma shape (e.g. Fig. 2C); 1, semi-elliptical (e.g. Fig. 2A).
(5) Size of the nuclear region (quantified as area of the nuclear region/area of the inner opercular region): 0, large (1/3 to 1) (e.g. Fig. 3F); 1, small (0 to 1/3) (e.g. Fig. 3C).
(6) Nuclear surface: 0, smooth (e.g. Fig. 3C); 1, grains and veins (e.g. Fig. 3R).
(7) Thickened nuclear region: 0, absent (e.g. Fig. 4B); 1, present (e.g. Fig. 4C).
(8) Sandpaper-like layer in the nuclear region: 0, absent (e.g. Fig. 4F); 1, present (e.g. Fig. 4J).
(9) Marginal region: 0, thin (e.g. Fig. 3C); 1, thickened (e.g. Fig. 3J).
(10) Growth grooves on the interior surface: 0, absent (e.g. Fig. 3C); 1, present (e.g. Fig. 3J).
(11) Growth lamellae on the exterior surface: 0, absent (e.g. Fig. 5A); 1, present (e.g. Fig. 2P).
(12) Distinctive patterns on the enamel region: 0, absent (e.g. Fig. 4A); 1, present (e.g. Fig. 4P).
(13) Thickened enamel region near the inner operculum region: 0, absent (e.g. Fig. 4A); 1, present (e.g. Fig. 4R).
(14) Concentric veins surrounding the nuclear region: 0, absent (e.g. Fig. 4C); 1, present (e.g. Fig. 4A).
(15) Marginal region obviously differing from the enamel region in colour: 0, absent (e.g. Fig. 3G); 1, present (e.g. Fig. 3F).
(16) Width of the marginal region: 0, narrow (e.g. Fig. 3C); 1, wide (e.g. Fig. 3F).

RESULTS

As summarized in Table 2, almost all species within a genus share the same combination of the 16 character states, except for *Filopaludina luzonica*. Most genera can be distinguished based on their unique combination of character states. Just four combinations of

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Table 2. Opercular characters of the 25 viviparid genera studied (see text for description and states of each character).

	Characters															
Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Lioplacinae																
Campeloma	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Lioplax	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Viviparinae																
Amuropaludina	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Callinina	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Rivularia	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Tulotoma	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1
Viviparus	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0
Bellamyinae																
Angulyagra	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0
Anularya	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Bellamya	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Celetaia	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Cipangopaludina	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
*Filopaludina**	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
F. luzonica	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
Heterogen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Idiopoma	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0
Margarya	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Mekongia	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Neothauma	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Notopala	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0
Sinotaia	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Taia	0	0	0	0	0	1	0	1	0	0	0	0	1	0	1	1
Tchangmargarya	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0
Torotaia	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Trochotaia	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0
Ussuripaludina	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

The asterisk indicates that *Filopaludina* does not include *F. luzonica*.

genera (*Neothauma* and *Mekongia*, *Celetaia* and *Torotaia*, *Angulyagra* and *Notopola*, *Sinotaia* and *Anularya*) share the same set of character states and cannot be identified based solely on the 16 opercular characters.

REVIEW OF OPERCULAR CHARACTERS

The opercular characters of the viviparid genera (and their constituent species) examined are presented in Table 2. Here, we provide a synopsis of the diagnostic characters for individual genera and example species, as well as formal changes of generic assignments. The number of specimens examined for each species is listed in Table 1.

Subfamily LIOPLACINAE Gill, 1863

Campeloma Rafinesque, 1819

Examined species: *Campeloma decisum* (Say, 1817) (Figs 2B, 3B, 6B).

Remarks: The elongated, inverted-comma-shaped and concentric operculum is the most important combination of diagnostic opercular characters for *Campeloma*. The apex–nucleus distance is clearly longer than the base–nucleus distance. This character is seen only in *Campeloma* and *Lioplax*, the two extant genera of Lioplacinae.

Lioplax Troschel, 1857

Examined species: *Lioplax subcarinata* (Say, 1817) (Figs 2A, 3A, 6A).

Remarks: *Lioplax* is the only genus in the family with a paucispiral operculum at early developmental stages, which is different from the concentric operculum of all other genera of Viviparidae. In addition, the semi-elliptical shape and lower central location of the nucleus are also diagnostic opercular characters for this genus.

Subfamily VIVIPARINAE Gray, 1847

Amuropaludina Moskvicheva, 1979

Examined species: *Amuropaludina praerosa* (Gerstfeldt, 1859) (Figs 2H, 3H, 6H).

Remarks: Within Viviparidae, *Amuropaludina* is the only genus with an operculum that has a central nucleus. All other genera of Viviparidae have an operculum with a subcentral nucleus. The wide semi-elliptical shape of *Amuropaludina* is also a useful diagnostic character.

Callinina Thiele, 1931

Examined species: *Callinina georgiana* (Lea, 1834) (Figs 2C, D, 3C, D, 6C, D); *C. intertexta* (Say, 1829) (Figs 2E, 3E, 6E).

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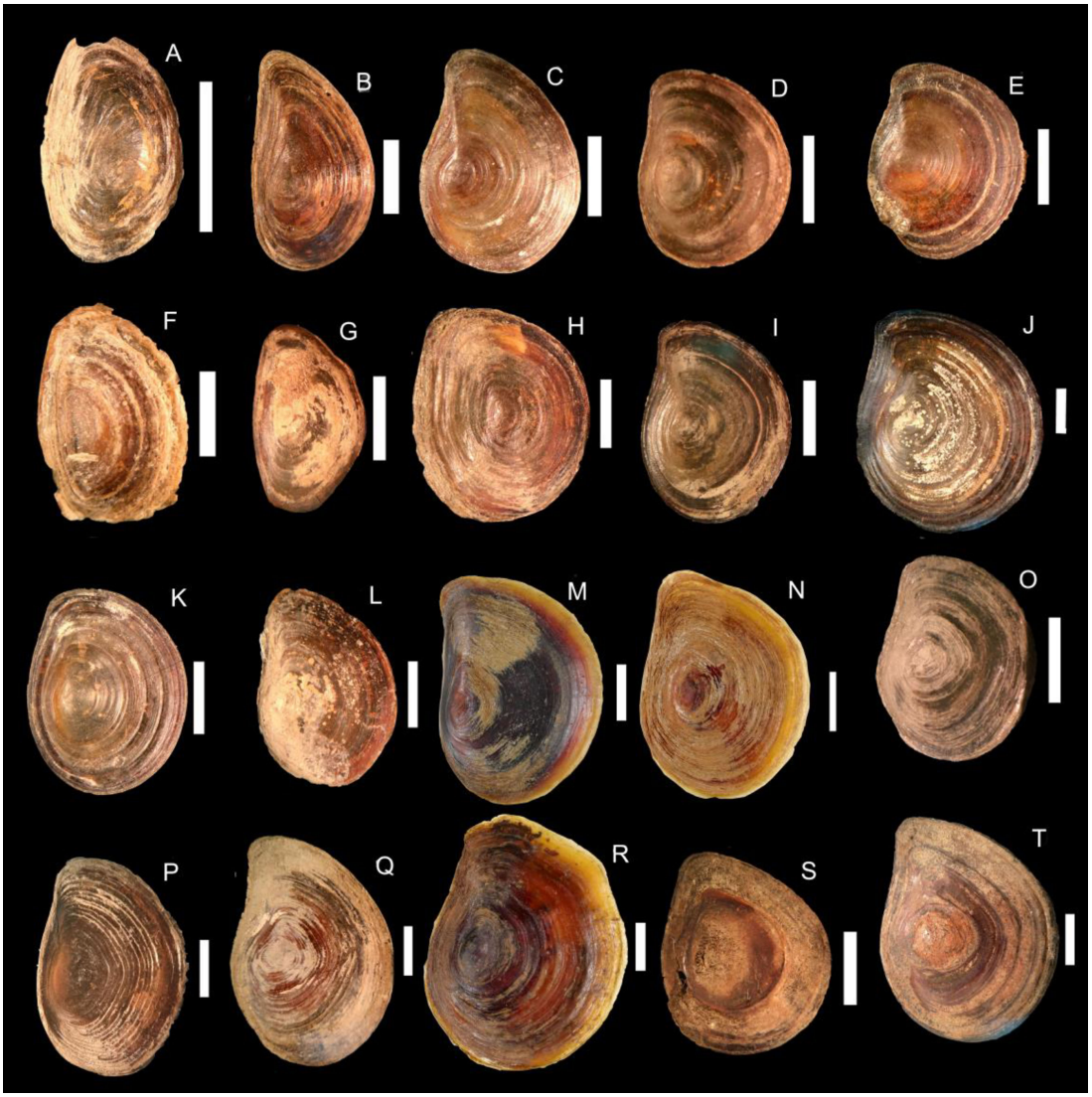


Figure 2. Exterior surface of opercula of the viviparid species studied. **A.** *Lioplax subcarinata*. **B.** *Campeloma decisum*. **C, D.** *Callinina georgiana*. **E.** *Callinina intertexta*. **F.** *Tulotoma magnifica*. **G.** *Rivularia auriculata*. **H.** *Amuropaludina praerosa*. **I.** *Viviparus viviparus*. **J, K.** *Viviparus contectus*. **L.** *Mekongia jullieni*. **M.** *Tchangmargarya multilabiata*. **N.** *Tchangmargarya yangtsunghaiensis*. **O.** *Cipangopaludina nagaensis*. **P.** *Cipangopaludina crassibuca*. **Q.** *Cipangopaludina chinensis*. **R.** *Margarya melanioides*. **S.** *Heterogen longispira*. **T.** *Heterogen japonica*. Scale bar: 5 mm.

Remarks: *Callinina* can be distinguished from *Viviparus* and the other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: a thin marginal region that obviously differs in colour from the enamel region; a shiny, smooth and relatively small nuclear region; and a narrow inner enamel region.

The only opercular difference found between the two examined species was a relatively darker and thicker operculum in the single examined individual of *C. intertexta* as compared with *C. georgiana*.

Rivularia **Heude, 1890**

Examined species: *Rivularia auriculata* (Martens, 1875) (Figs 2G, 3G, 6G).

Remarks: The semi-elliptical operculum of *Rivularia* is most similar to that of *Tulotoma*. However, the operculum of *Rivularia* has no obvious marginal region; there is, however, a smooth, large scar region.

Tulotoma **Haldeman, 1840**

Examined species: *Tulotoma magnifica* (Conrad, 1834) (Figs 2F, 3F, 6F).

Remarks: The operculum of *Tulotoma* can be easily distinguished from that of all other genera based on the following characters: semi-elliptical shape; inwardly folded opercular apex and opercular base; outwardly folded inner margin; a rough, non-transparent and small inner opercular region; and a wide, thin marginal region that obviously differs in colour from the enamel

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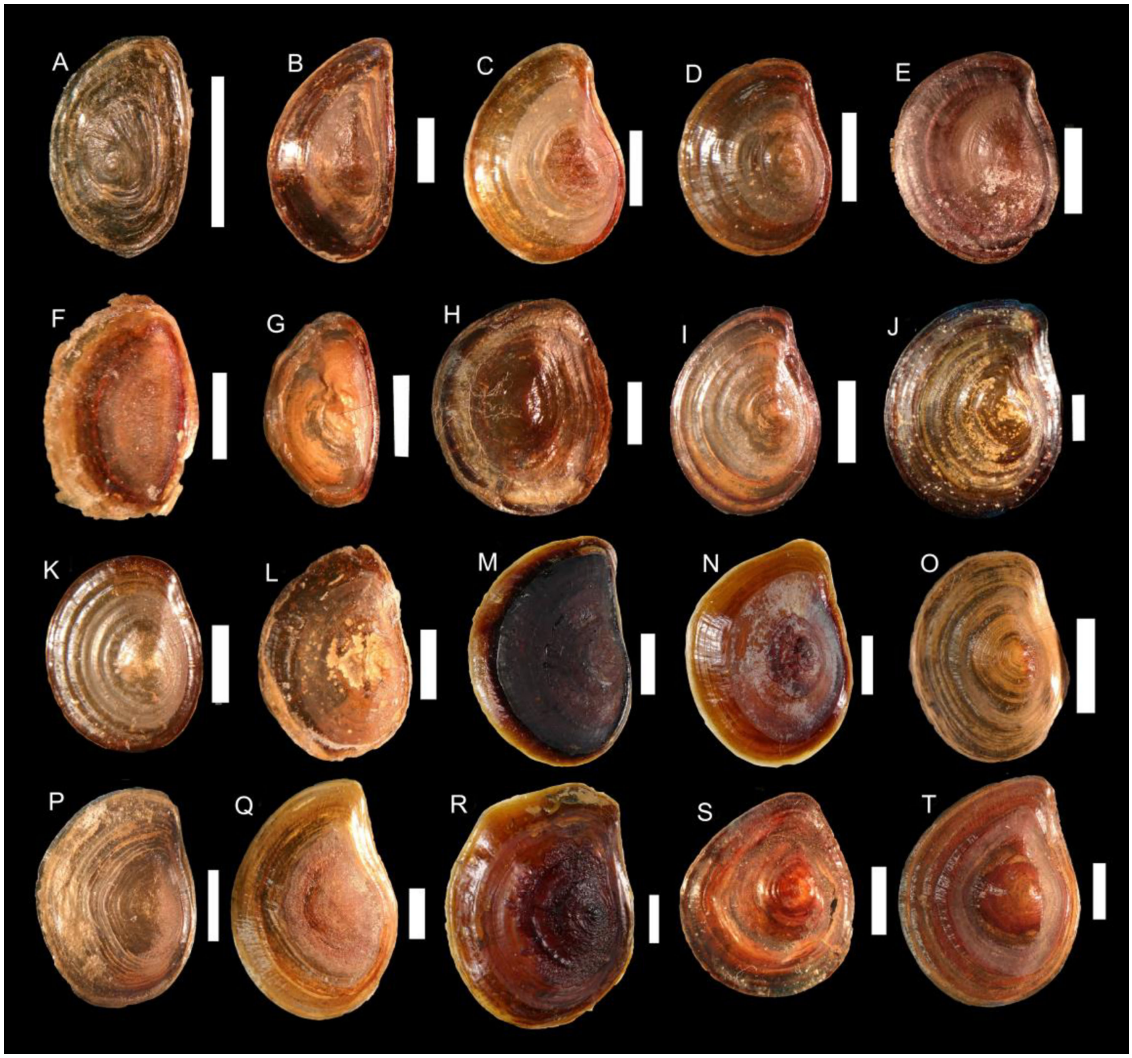


Figure 3. Interior surface of opercula of the viviparid species studied. **A.** *Lioplax subcarinata*. **B.** *Campeloma decisum*. **C, D.** *Callinina georgiana*. **E.** *Callinina intertexta*. **F.** *Tulotoma magnifica*. **G.** *Rivularia auriculata*. **H.** *Amuropaludina praerosa*. **I.** *Viviparus viviparus*. **J, K.** *Viviparus contectus*. **L.** *Mekongia jullieni*. **M.** *Tchangmargarya multilabiata*. **N.** *Tchangmargarya yangtsunghaiensis*. **O.** *Cipangopaludina nagaensis*. **P.** *Cipangopaludina crassibuca*. **Q.** *Cipangopaludina chinensis*. **R.** *Margarya melanioides*. **S.** *Heterogen longispira*. **T.** *Heterogen japonica*. Scale bar: 5 mm.

region, especially when it is compared with *Rivularia* Heude, 1890. Like *Tulotoma*, *Rivularia* has a semi-elliptical shape and subcentral nucleus.

Viviparus Montfort, 1810

Examined species: *Viviparus viviparus* (Linnaeus, 1758) (Figs 2I, 3I, 6I); *V. contectus* Millet, 1813 (Figs 2J, K, 3J, K, 6J, K).

Remarks: *Viviparus* can be distinguished from the other genera with a concentric inverted-comma-shaped operculum (especially *Callinina*) by the following combination of characters: a thickened marginal region, obvious concentric growth grooves on the interior surface, a wide inner enamel region, and a small and smooth nuclear region.

The operculum of *V. contectus* differs from that of *V. viviparus* in having a thicker marginal region and more obvious grooves.

Subfamily BELLAMYINAE Rohrbach, 1937

Angulyagra Rao, 1931

Examined species: *Angulyagra* cf. *polyzonata* (Frauenfeld, 1862) (Figs 4T, 5T, 7T).

Remarks: Apart from *Notopala*, *Angulyagra* can be distinguished from other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: a relatively small nuclear region with shiny grains or veins, a relatively thick enamel layer and a thin marginal region that obviously differs in colour from the enamel region.

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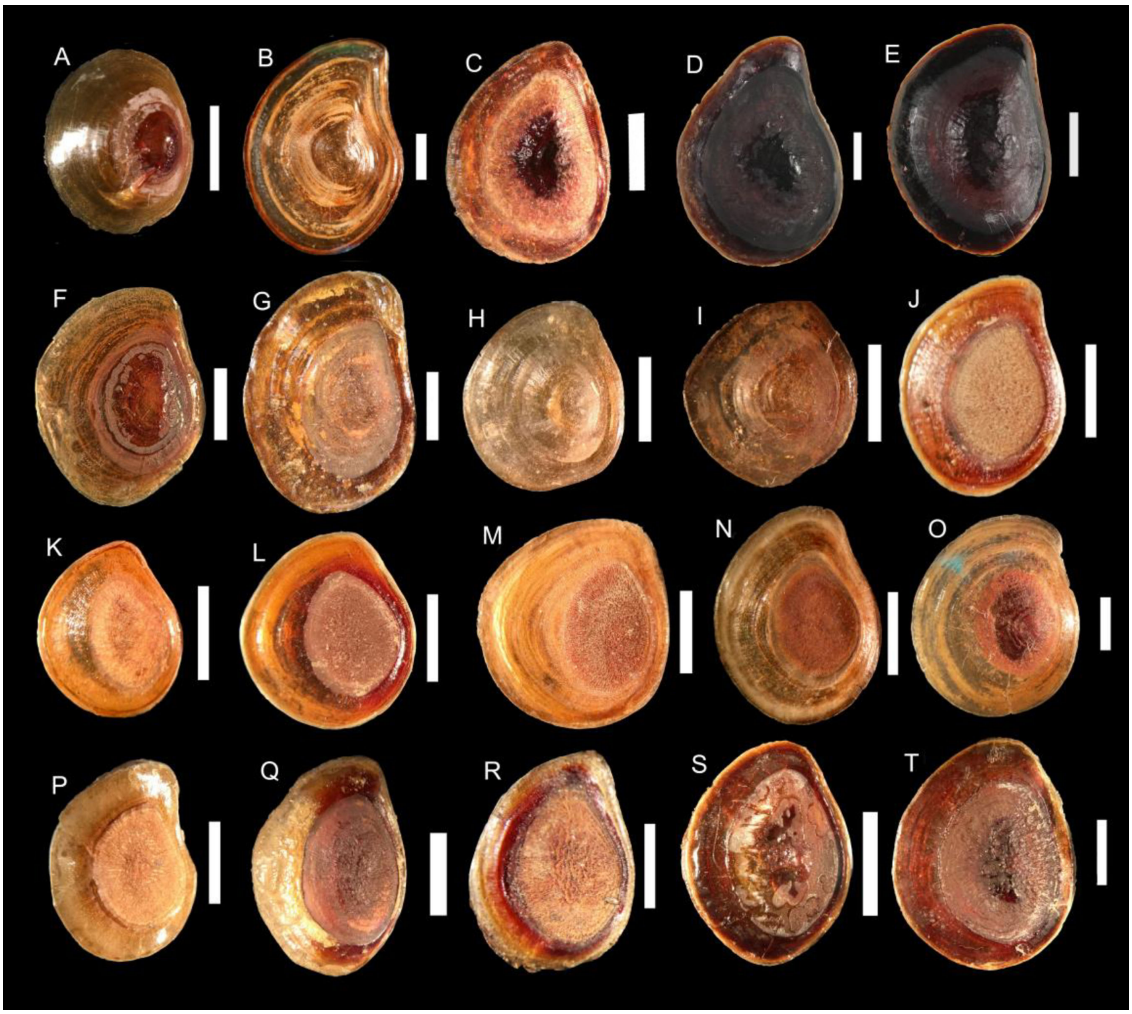


Figure 4. Interior surface of opercula of the viviparid species studied. **A.** *Celetaia persculpta*. **B.** *Ussuripaludina ussuriensis*. **C.** *Sinotaia angularis*. **D.** *Anularya mansuyi*. **E.** *Anularya bicostata*. **F.** *Torotaia mainitensis*. **G.** *Neothauma tanganyicense*. **H.** *Bellamya phthinotropis*. **I.** *Bellamya trochlearis*. **J.** *Idiopoma umbilicata*. **K.** *Idiopoma dissimilis*. **L.** *Filopaludina luzonica*. **M.** *Filopaludina martensi*. **N.** *Filopaludina bengalensis*. **O.** *Filopaludina gigantea*. **P.** *Trochotaia trochoides*. **Q.** *Taia naticoides*. **R.** *Taia shanensis*. **S.** *Notopala ampullaroides*. **T.** *Angulyagra* cf. *polyzonata*. Scale bar: 5 mm.

One species of *Angulyagra* (total of three specimens with operculum) has been examined for this study (Table 1).

Anularya Zhang & Chen, 2015

Examined species: *Anularya mansuyi* (Dautzenberg & Fischer, 1905) (Figs 4D, 5D, 7D); *A. bicostata* (Tchang & Tsi, 1949) (Figs 4E, 5E, 7E).

Remarks: Except for the larger overall size and inner opercular region, the operculum of *Anularya* is most similar to that of *Sinotaia*. Apart from *Sinotaia*, *Anularya* can be distinguished from other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: a thick, smooth, shiny, nontransparent and small nuclear region and a thick enamel region.

The operculum of *A. mansuyi* differs from that of *A. bicostata* by having a more acute opercular apex. The thickness of the adult operculum of *A. mansuyi* can vary from relatively thin to thick. Therefore, its colour can be transparent orange red to relatively nontransparent dark red.

Bellamya Jousseaume, 1886

Examined species: *Bellamya phthinotropis* (Martens, 1892) (Figs 4H, 5H, 7H); *B. trochlearis* Martens, 1892 (Figs 4I, 5I, 7I).

Remarks: *Bellamya* can be distinguished from other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: opercular height almost as same as width; a small, smooth shiny nuclear region; and a thin and narrow marginal region.

The operculum of *B. trochlearis* differs from that of the other species examined, *B. phthinotropis*, by having a more notably convex outer margin—this fits the shape of the aperture and has a strong keel.

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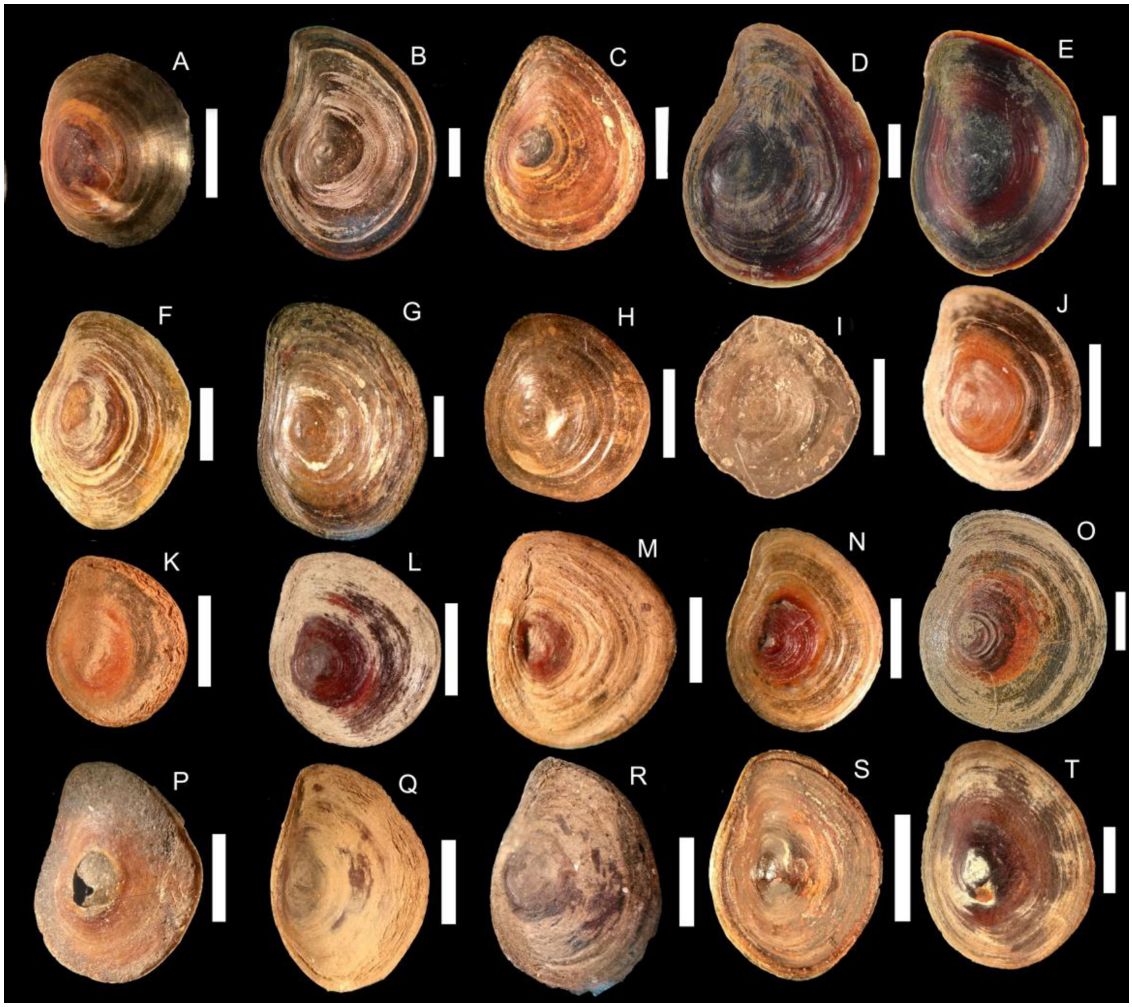


Figure 5. Exterior surface of opercula of the viviparid species studied. **A.** *Celetaia persculpta*. **B.** *Ussuripaludina ussuriensis*. **C.** *Sinotaia angularis*. **D.** *Anularya mansuyi*. **E.** *Anularya bicostata*. **F.** *Torotaia mainitensis*. **G.** *Neothauma tanganyicense*. **H.** *Bellamya phthinotropis*. **I.** *Bellamya trochlearis*. **J.** *Idiopoma umbilicata*. **K.** *Idiopoma dissimilis*. **L.** *Filopaludina luzonica*. **M.** *Filopaludina martensi*. **N.** *Filopaludina bengalensis*. **O.** *Filopaludina gigantea*. **P.** *Trochotaia trochoides*. **Q.** *Taia naticoides*. **R.** *Taia shanensis*. **S.** *Notopala ampullaroides*. **T.** *Angulyagra* cf. *polyzonata*. Scale bar: 5 mm.

Celetaia Clench, 1966

Examined species: *Celetaia persculpta* (Sarasin & Sarasin, 1898) (Figs 4A, 5A, 7A).

Remarks: Apart from *Torotaia*, *Celetaia* can be distinguished from other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: a blunt opercular apex; a large, smooth and shiny nuclear region, sometimes surrounded by concentric veins; and a thin marginal region.

Cipangopaludina Hannibal, 1912

Examined species: *Cipangopaludina chinensis* (Gray, 1834); *C. nagaensis* (Preston, 1914) n. comb.; *C. crassibuca* (Sarasin & Sarasin, 1898).

Remarks: The *Cipangopaludina* species examined have a similar operculum, which can be distinguished from the other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: regular growth lamellae on the exterior surface, a relatively small inner operculum region and a large nuclear region with a sandpaper-like layer (sometimes partly absent).

Cipangopaludina chinensis (Gray, 1834)
(Figs 2Q, 3Q, 6Q)

Remarks: This species has a large nuclear region with a sandpaper-like layer (nontransparent); this layer is sometimes partly or even completely absent in the nuclear region of some individuals, especially juveniles.

Cipangopaludina nagaensis (Preston, 1914) new combination
(Figs 2O, 3O, 6O)

Vivipara nagaensis Preston, 1914: 20 (Naga Hills, India).

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Figure 6. Shells of the viviparid species studied. **A.** *Lioplax subcarinata*. **B.** *Campeloma decisum*. **C, D.** *Callinina georgiana*. **E.** *Callinina intertexta*. **F.** *Tulotoma magnifica*. **G.** *Rivularia auriculata*. **H.** *Amuropaludina praerosa*. **I.** *Viviparus viviparus*. **J, K.** *Viviparus contectus*. **L.** *Mekongia jullieni*. **M.** *Tchangmargarya yangtsunghaiensis*. **N.** *Tchangmargarya multilabiata*. **O.** *Cipangopaludina nagaensis*. **P.** *Cipangopaludina crassibuca*. **Q.** *Cipangopaludina chinensis*. **R.** *Margarya melanioides*. **S.** *Heterogen longispira*. **T.** *Heterogen japonica*. Scale bar:10 mm.

Remarks: *Vivipara nagaensis* Preston, 1914 has been rarely mentioned in the literature after its description. Here, it is classified as a species of *Cipangopaludina* based on its shell morphology and the fact that it exhibits the generic characters of the *Cipangopaludina* operculum (i.e. as seen in the type species of the genus, *C. chinensis*). The shell and operculum of *C. nagaensis* are much smaller than those in *C. chinensis* and only a small part of the nuclear region in *C. nagaensis* is covered by the sandpaper-like layer.

Cipangopaludina crassibuca (Sarasin & Sarasin, 1898)
(Figs 2P, 3P, 6P)

Vivipara crassibuca Sarasin & Sarasin, 1898: 60, figs 120, 131, 132 (Lake Poso, Central Sulawesi, Indonesia).
Cipangopaludina crassibuca—Prashad, 1928: 176.
Idiopoma crassibucca—Schuh *et al.*, 2010: https://www.discoverlife.org/mp/20q?search=Idiopoma+crassibucca.

Remarks: *Vivipara crassibuca* Sarasin & Sarasin, 1898 has been rarely mentioned after its description. Prashad (1928) regarded it as undoubtedly belonging to *Cipangopaludina* based on shell morphology. Schuh *et al.* (2010) assigned this species to *Idiopoma* in their online database but provided no justification. Here, we assign it to

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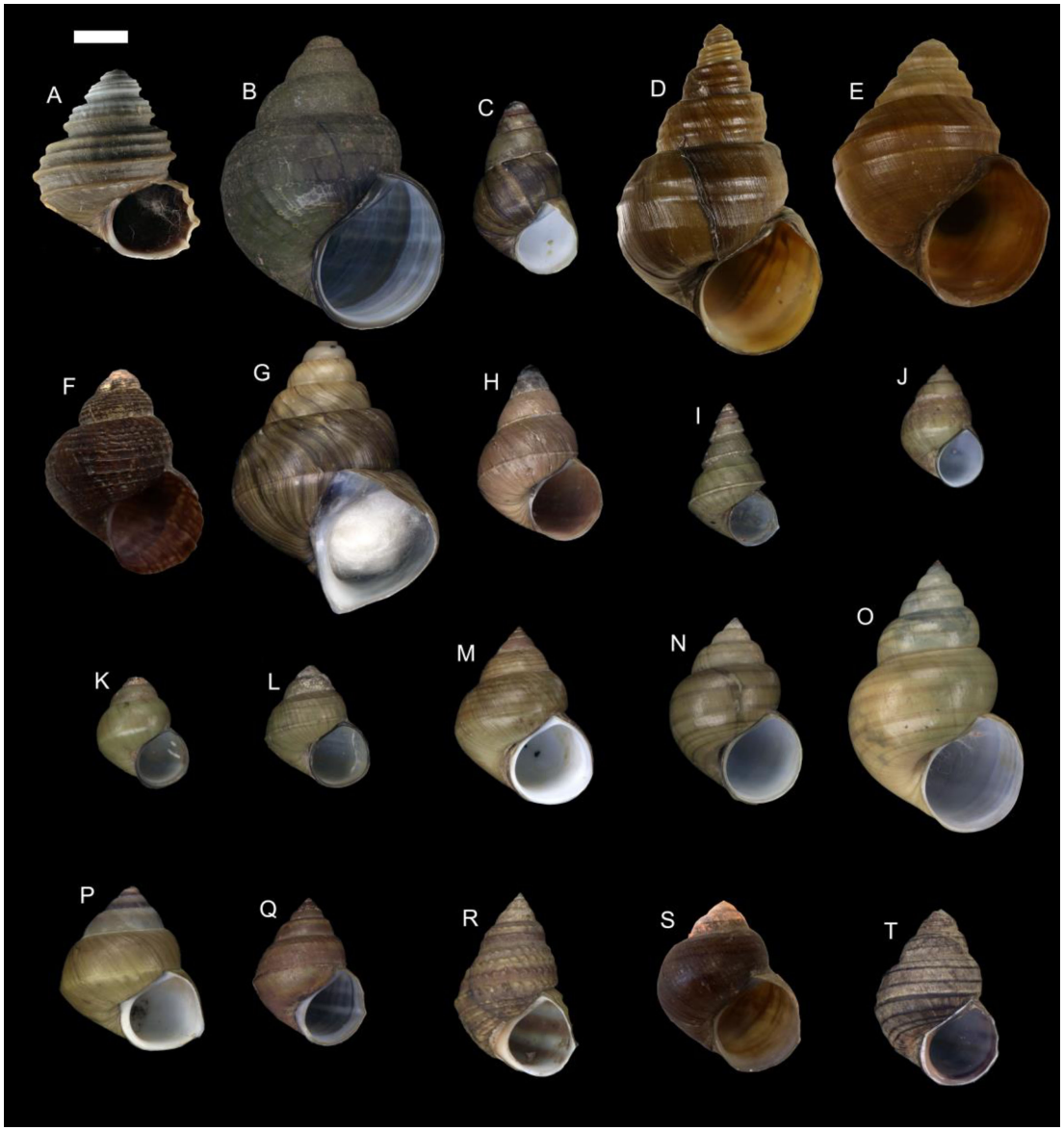


Figure 7. Shells of the viviparid species studied. **A.** *Celetaia persculpta*. **B.** *Ussuripaludina ussuriensis*. **C.** *Sinotaia angularis*. **D.** *Anularya mansuyi*. **E.** *Anularya bicostata*. **F.** *Torotaia mainitensis*. **G.** *Neothauma tanganyicense*. **H**. *Bellamya phthinotropis*. **I.** *Bellamya trochlearis*. **J.** *Idiopoma umbilicata*. **K.** *Idiopoma dissimilis*. **L.** *Filopaludina luzonica*. **M.** *Filopaludina martensi*. **N.** *Filopaludina bengalensis*. **O.** *Filopaludina gigantea*. **P.** *Trochotaia trochoides*. **Q.** *Taia naticoides*. **R.** *Taia shanensis*. **S.** *Notopala ampullaroides*. **T.** *Angulyagra* cf. *polyzonata*. Scale bar: 10 mm.

Cipangopaludina based on its shell morphology and the fact that its operculum exhibits the generic characters of *Cipangopaludina*. The operculum of *C. crassibuca* has denser growth lamellae compared to the other two species *of Cipangopaludina* examined. In addition, the convex inner margin of the first species is not as pronounced as in the other two species.

***Filopaludina* Habe, 1964**

Examined species: *Filopaludina martensi* (Frauenfeld, 1864) (Figs 4M, 5M, 7M); *Filopaludina bengalensis* (Lamarck, 1822) (Figs 4N, 5N, 7N); *Filopaludina gigantea* (von dem Busch, 1843) (Figs 4O, 5O, 7O); *Filopaludina luzonica* (Kobelt, 1909) (Figs 4L, 5L, 7L).

Remarks: The opercular characters of *Filopaludina* are broadly similar to those of *Idiopoma*. Notably, both show an inner opercular region almost completely covered by a sandpaper-like layer. However, in *Filopaludina*, this layer and the inner opercular region are maroon coloured, whereas in *Idiopoma* they are brownish yellow. In addition, the enamel region of *Filopaludina* lacks the fine grid-like pattern found in the enamel region of *Idiopoma*.

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The operculum of *F. martensi* differs from that of the other examined species of *Filopaludina*: it has a relatively small inner opercular region that is almost completely covered by a sandpaper-like layer, with many radial veins; is almost as high as it is wide; and has a notably convex outer margin.

The opercula of *F. bengalensis* and *F. gigantea* are similar, but the former is much smaller (as is the shell). *Filopaludina gigantea* has been considered as a large variety of *F. bengalensis*. The specimen of *F. gigantea* examined partly lacks the sandpaper-like layer and instead exhibits a smooth maroon nuclear region that, if confirmed as being present in more specimens, might be a size-independent character for distinguishing the two species.

The operculum of *F. luzonica* stands out because of the conspicuous difference in colour between the marginal and enamel regions. This character is absent in the other *Filopaludina* species examined but is present in other genera, such as *Idiopoma* and *Taia* (Table 2).

Heterogen Annandale, 1921

Examined species: *Heterogen longispira* (E. A. Smith, 1886) (Figs 2S, 3S, 6S); *Heterogen japonica* (Martens, 1861) (Figs 2T, 3T, 6T).

Remarks: *Heterogen longispira* has always been considered as the only species of the genus *Heterogen*, which is endemic to Lake Biwa, Japan. Recently, '*Cipangopaludina*' *japonica* has also been assigned to the genus on the basis of molecular systematic data (Hirano *et al.*, 2019a); the opercular characters of this species also match those of *H. longispira*, including a relatively large, smooth and shiny nuclear region and a thin marginal region. The combination of these opercular characters differs from those found in other viviparid genera with a concentric inverted-comma-shaped operculum.

The operculum of *H. japonica* is clearly higher than it is wide, while in *H. longispira* the opercular height and width are roughly the same. In addition, *H. longispira* has a larger inner opercular region as compared with *H. japonica*.

Idiopoma Pilsbry, 1901

Examined species: *Idiopoma umbilicata* (Lea, 1856) (Figs 4J, 5J, 7J); *Idiopoma dissimilis* (Müller, 1774) (Figs 4K, 5K, 7K).

Remarks: *Idiopoma* can be distinguished from other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: a whole inner opercular region covered by a thick, rough, brownish-yellow sandpaper-like layer; a thin marginal region obviously differing from the enamel region in colour; and an enamel region that is smooth but has a fine grid-like pattern.

The operculum of *I. dissimilis* is about as high as it is wide, while in *I. umbilicata* the operculum is distinctly higher than it is wide. This difference can be attributed to differences in aperture shape between the two species.

Margarya Nevil, 1877

Examined species: *Margarya melanioides* (Dautzenberg & Fischer, 1905) (Figs 2R, 3R, 6R).

Remarks: *Margarya* can be distinguished from other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: a large nuclear region with many grains and veins, and a large inner opercular region.

Mekongia Crosse & P. Fischer, 1876

Examined species: *Mekongia jullieni* (Deshayes, 1876) (Figs 2L, 3L, 6L).

Remarks: *Mekongia* is not a monophyletic group according to two recent molecular systematic studies (Hirano *et al.*, 2019a; Stelbrink *et al.*, 2020). Here, we only examined the type species of *Mekongia*, *M. jullieni*. *Mekongia* can be distinguished from the other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: a nucleus very close to the inner margin, an inner enamel region that is very narrow in width, a large inner opercular region and a relatively small nuclear region with fine shiny grains.

Neothauma E. A. Smith, 1880

Examined species: *Neothauma tanganyicense* E. A. Smith, 1880 (Figs 4G, 5G, 7G).

Remarks: Apart from *Mekongia*, the monotypic genus *Neothauma* can be distinguished from other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: height clearly larger than width and a small nuclear region that is relatively rough, with many shiny grains or veins.

Notopala Cotton, 1935

Examined species: *Notopala ampullaroides* (Reeve, 1863) (Figs 4S, 5S, 7S).

Remarks: Apart from *Angulyagra*, *Notopala* can be distinguished from other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: a relatively small nuclear region with shiny grains or veins, a relatively thick enamel layer and a thin marginal region that obviously differs in colour from the enamel region.

Sinotaia Haas, 1939

Examined species: *Sinotaia angularis* (Müller, 1774) (Figs 4C, 5C, 7C).

Remarks: Apart from *Anularya*, *Sinotaia* can be distinguished from other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: a thick, smooth, shiny, non-transparent and small nuclear region and a thick enamel region.

Taia Annandale, 1918

Examined species: *Taia naticoides* (Kobelt, 1909) (Figs 4Q, 5Q, 7Q); *Taia shanensis* (Kobelt, 1909) (Figs 4R, 5R, 7R).

Remarks: *Taia* can be distinguished from other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: a subcentral nucleus that is very close to the inner margin; a large nuclear region with many fine, dense grains or veins; an inner opercular region completely or partly covered by a rough sandpaper-like layer; an enamel region that is thickened and deeper in colour near the inner opercular region; and a thin, wide marginal region that obviously differs in colour from the enamel region.

The inner nuclear region of *T. shanensis* is almost completely covered by a thick sandpaper-like layer, which is mostly absent in *T. naticoides*.

Tchangmargarya He, 2013

Examined species: *Tchangmargarya yangtsunghaiensis* (Tchang & Tsi, 1949) (Figs 2N, 3N, 6M); *Tchangmargarya multilabiata* (Zhang & Chen, 2015) (Figs 2M, 3M, 6N).

Remarks: The two species of *Tchangmargarya* examined show the largest opercular differences within one genus of Viviparidae. This

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is especially true of the position of the nucleus. Nevertheless, these two species can still be assigned to a single genus and distinguished from other genera with a concentric inverted-comma-shaped operculum by the following combination of characters: a nuclear region with many grains or veins, an enamel region that is thickened near the inner opercular region and deeper in colour, and a thin marginal region obviously differing in colour from the enamel region.

The operculum of *T. multilabiata* is obviously different from that of *T. yangtsunghaiensis*. In the former, the nucleus is positioned close to the inner margin and inner opercular region is larger; while the outer inner and upper inner margins of *T. yangtsunghaiensis* are inwardly curved, in *T. multilabiata* only the upper inner margin is inwardly curved.

***Torotaia* Haas, 1939**

Examined species: *Torotaia mainitensis* (Bartsch, 1907) (Figs 4F, 5F, 7F).

Remarks: The operculum of *Torotaia* is distinct from that of most genera, but similar to that of *Celetaia* in the following characters: a large, smooth and shiny nuclear region, which is sometimes surrounded by concentric veins, and a thin marginal region. However, the operculum of *Torotaia* is more fragile and thinner, with the apex being noticeably more acute than in *Celetaia*.

***Trochotaia* Brandt, 1974**

Examined species: *Trochotaia trochoides* (Martens, 1860) (Figs 4P, 5P, 7P).

Remarks: *Trochotaia* can be distinguished from the other genera by a concentric inverted-comma-shaped operculum that exhibits the following combination of characters: a thick, rough layer with many grains or veins over the whole of the inner opercular region; a clearly convex outer margin; and a smooth enamel region with a dense ray-like pattern.

***Ussuripaludina* Zatravkin & Bogatov, 1987**

Examined species: *Ussuripaludina ussuriensis* (Gerstfeldt, 1859) (Figs 4B, 5B, 7B).

Remarks: *Ussuripaludina* can be distinguished from the other viviparid genera studied by a concentric inverted-comma-shaped operculum that shows the following combination of characters: a shiny, smooth and convex nuclear region; a shiny and smooth scar region; a relatively large inner opercular region; and a narrow marginal region.

DISCUSSION

Our results show that, when studied in detail and a standardized approach is used, operculum characters can provide useful taxonomic information in Viviparidae. Most previous studies on Viviparidae only focused on the general shape of the operculum and the position of the nucleus on the exterior surface (e.g. Van Damme & Pickford, 1998; Lu *et al.*, 2014). However, we found that the interior surface of the operculum provides more diagnostic characters and this is especially true for the nuclear, enamel and marginal regions.

Viviparidae show little intraspecific variation in their opercular characters. The opercula of conspecifics varying in size (e.g. *Viviparus contectus*; Fig. 6J, K) or colour pattern (e.g. *Callinina georgiana*; Fig. 6C, D) generally have almost identical diagnostic characters (Figs 2J, K, C, D, 3J, K, C, D). However, some characters, such as the sandpaper-like layer covering the inner opercular region of *Cipangopaludina* species, can vary within species. The uniformity of opercular features within individual species coupled with the presence of specific opercular traits that consistently differ between species made it possible for us to clearly discriminate between the majority of species examined solely on the basis of opercular characters (34 of 38 comparisons; the exceptions were *Filopaludina bengalensis* and *F. gigantea*, and *Taia naticoides* and *T. shanensis*). Therefore, we consider that opercular characters can be employed as informative characters for species identification in addition to shell and anatomical characters.

As we have shown, the opercular characters described here are also useful for genus-level identification of Viviparidae. Based on the examination of 25 out of a total of 28 viviparid genera, we found that opercular characters are more conservative within a genus and display more obvious differences between genera than does shell morphology. One typical example is the genus *Heterogen*, formerly considered endemic to Lake Biwa, Japan. *Heterogen longispira* was always considered as the only species in this genus. Its embryonic shell with a flat shoulder obviously differs from that of '*Cipangopaludina*' *japonica*, which lacks a shoulder (Fig. 5S, T). In addition, the acute shell apex of '*Cipangopaludina*' *japonica* is quite similar to that of other species of *Cipangopaludina* (Fig. 5O, P, Q). However, according to a recent molecular systematic study, *H. longispira* falls within the '*Cipangopaludina*' *japonica* clade (Hirano *et al.*, 2015). This clade does not cluster with other species of *Cipangopaludina* but is closely related to *Ussuripaludina* and *Celetaia* (Hirano *et al.*, 2019a; Stelbrink *et al.*, 2020). Consequently, Hirano *et al.* (2019a) used the combination *Heterogen japonica* instead of *Cipangopaludina japonica* in their molecular phylogenetic study. A recent study by Zhang *et al.* (2015) has also shown that identification based on embryonic shell morphology could lead to wrong genus-level assignments in Viviparidae. The opercular characters of '*Cipangopaludina*' *japonica* are broadly similar to those of *H. longispira* and distinct from those of all other examined *Cipangopaludina* species, thus fitting the molecular results and demonstrating the usefulness of the operculum for delimiting genera.

The potential of opercular characters to outperform shell characteristics at genus-level taxonomy is underlined by the fact that the use of the former allows clear separation of several valid viviparid genera that on the basis shell morphology have traditionally been considered as junior synonyms. *Amuropaludina* and *Callinina* have been considered as junior synonyms of *Viviparus* and were ignored by most researchers. Similarly, *Ussuripaludina* was considered as a junior synonym of *Cipangopaludina*. Several recent molecular systematic studies have confirmed the validity of these genera (Hirano *et al.*, 2019a; Stelbrink *et al.*, 2020). Similarly, species of *Anularya* and *Tchangmargarya* have been assigned to *Margarya* due to their thick and obviously sculptured shells. Plesiomorphic shell characters (such as the similar smooth and round shell of *Viviparus*, *Callinina* and *Amuropaludina*, all of which belong to Viviparinae; Fig. 5C, H, I) and convergence of shell morphology (e.g. shouldered, thick and sculptured shells of *Anularya*, *Tchangmargarya* and *Margarya*; Figs 5M, R, 7D) may have led to the previous misclassification. However, all genera mentioned above can be easily differentiated solely based on opercular characters (Table 2).

Congeneric species usually have similar opercula and share some putative synapomorphies, such as the growth grooves found on the interior opercular surface in *Viviparus* (these are absent in *Callinina*) and the growth lamellae on the exterior opercular surface of *Cipangopaludina* (absent in *Ussuripaludina*). Zhang *et al.* (2015) and Zhang (2017) systematically studied the opercula of all extant species of *Anularya*, *Tchangmargarya* and *Margarya*, showing the genus-specific opercular characters of these three genera. The differences in opercula between congeners usually concern the shape and size of the operculum. Some genera, such as *Tchangmargarya* and *Filopaludina*, can display a higher variation of opercular characters between different congeners. The combination of opercular characters in Table 2 can be used for genus-level taxonomy in most cases; the exceptions are *Neothauma/Mekongia*, *Celetaia/Torotaia*, *Angulyagra/Notopala* and *Sinotaia/Anularya*. Based on opercular characters alone, these genera can only be distinguished by differences in the shape and size of the operculum.

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Here, we examined roughly a quarter (38) of the *c.* 125–150 viviparid species considered valid. Some genera with relatively high diversity, such as *Campeloma*, *Bellamya* and *Cipangopaludina*, deserve further study to confirm the genus-specific combination of character states identified here. Nonetheless, our study clearly demonstrates that the opercular characters of Viviparidae can be used as important diagnostic characters for recognizing species and genera. Opercular characters should be routinely included in addition to shell morphology and anatomy in future morphology-based taxonomic and systematic studies. In this way, a more complete and reliable database for the morphological identification and taxonomy of viviparid taxa can be built.

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PENSOFT

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A new genus of river snails, *Dalipaludina* (Gastropoda, Viviparidae), endemic to the Yunnan Plateau of SW China

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Abstract

A new genus of viviparid snail, *Dalipaludina* **gen. nov.**, from the Yunnan Plateau of China is described within an integrative taxonomic framework based on data from the mitochondrial COI marker and morphology. *Dalipaludina* can be distinguished from all other viviparid genera by a unique combination shell, operculum and radula characters. Four species are assigned here to the new genus, *Dalipaludina delavayana* **comb. nov.**, *Dalipaludina oxytropoides* **comb. nov.**, *Dalipaludina occidentalis* **comb. nov.**, and *Dalipaludina pyramidella* **comb. nov.**, and one species is newly assigned to *Margarya*, *Margarya dianchiensis* **comb. nov.** The four species of *Dalipaludina* are allopatrically distributed in shallow water lentic habitats at high altitude regions of the Yunnan Plateau.

Key Words

High altitude, lentic habitat, phylogeny, taxonomy

Introduction

River snails (Viviparidae) are widely distributed freshwater gastropods that are found on all continents except for Antarctica and South America. The oldest fossil record of Viviparidae can be traced back to the mid Jurassic. About 28 extant genera and 125 to 150 extant species of this family worldwide are currently recognised (Van Bocxlaer and Strong 2019). China is the country with the highest biodiversity of Viviparidae, harbouring two subfamilies (Bellamyinae Rohrbach, 1937; Viviparinae J. E. Gray, 1847), 13 genera (*Amuropaludina* Moskvicheva, 1979; *Angulyagra* Rao, 1931; *Anularya* Zhang & Chen, 2015; *Cipangopaludina* Hannibal, 1912; *Filopaludina* Habe, 1964; *Idiopoma* Pilsbry, 1901; *Margarya* G. Nevill, 1877; *Mekongia* Crosse & P. Fischer, 1876 *Rivularia* Heude, 1890; *Sinotaia* F. Haas, 1939; *Tchangmargarya* He, 2013; *Ussuripaludina* Zatravkin & Bogatov, 1987; *Viviparus* Montfort, 1810), and at least 40 species (Liu et al 1995; Zhang et al. 2015; Van Bocxlaer et al. 2017).

The Yunnan Province of China, which covers an area of only 394,000 km^2, harbours nine genera (*Angulyagra*; *Anularya*; *Cipangopaludina*; *Filopaludina*; *Idiopoma*; *Margarya*; *Mekongia*; *Sinotaia*; *Tchangmargarya*) including three endemic ones (*Anularya*; *Margarya*; *Tchangmargarya*) and at least 24 species including 14 endemic ones of Viviparidae (Liu et al 1995; Zhang et al. 1997; Zhang et al. 2015). While it is the region with the highest diversity of Viviparidae at both the genus and species level not only in China but also worldwide, recent studies (Du et al. 2011; Zhang et al. 2015; Zhang 2017) have revealed that the diversity of Viviparidae in Yunnan is still underestimated. Based on an integrative study combining molecular and morphological data, we here revise the taxonomy of several viviparid species from the Yunnan Plateau, and describe a new genus. The phylogeny and ecology of the new genus, as well as conservation aspects, are discussed.

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Materials and methods

Material

Type specimens of three Viviparidae species from Yunann kept in the Institute of Zoology, Chinese Academy of Sciences, Beijing (**IZCAS**), the Kunming Natural History Museum of Zoology, Kunming (**KIZ**), and the Museum of Comparative Zoology at Harvard University, Cambridge (**MCZ**), as well as other specimens in Museum für Naturkunde, Berlin (**ZMB**), have been examined. From 2011 to 2022, altogether 19 lakes of Yunnan and the surrounding aquatic habitats, including rivers, creeks, springs and wetlands, have been surveyed (Fig. 1A). Newly collected fresh samples of the species belonging to the new genus were collected by Le-Jia Zhang and Jiao-Wei Ning from 2018 to 2022 in northwest Yunnan (Fig. 1B–D). The newly sampled material is stored in ZMB and the private collection of Le-Jia Zhang (**ZLJ**) Jiao-Wei Ning (**NJW**).

Examination of morphology

Shell height (H) and width (W) of only mature and complete specimens were measured with a caliper to a precision of 0.1 mm (Table 1). The morphometric data of

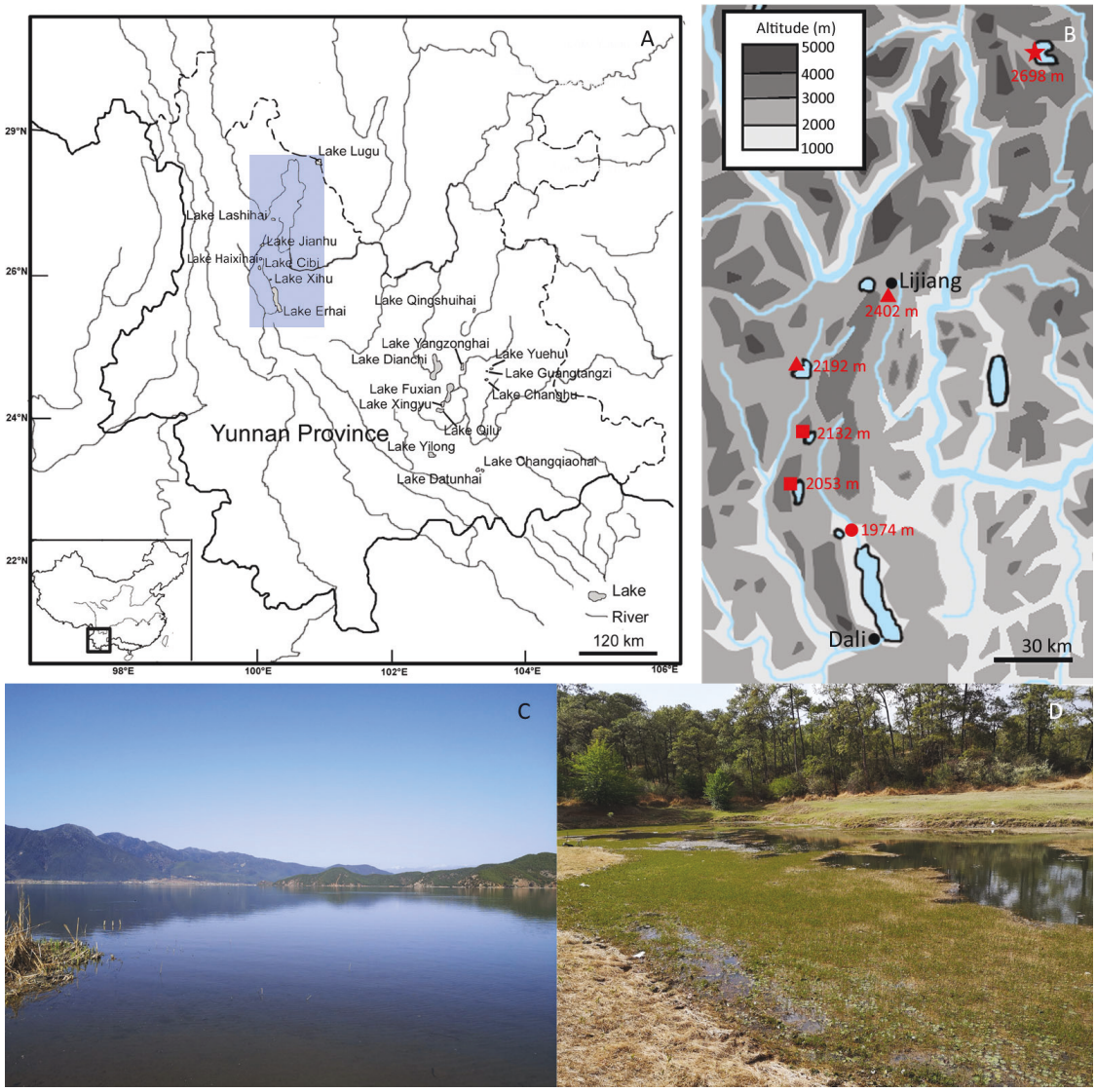


Figure 1. Collecting sites and habitats of *Dalipaludina* gen. nov. in Yunnan, China. **A.** Map of Yunnan Province showing 19 surveyed lakes (adapted from Zhang et al. 2015), study area in northwest Yunnan highlighted in blue, where *Dalipaludina* species were found during surveys; **B.** Map of study area in northwest Yunnan, star–*Dalipaludina oxytropoides*, triangle–*Dalipaludina occidentalis*, square–*Dalipaludina delavayana*, circle–*Dalipaludina pyramidella*; altitude of each collecting site is given; **C.** Environment of one collecting site of *Dalipaludina oxytropoides* in Lake Lugu; **D.** Environment of one collecting site of *Dalipaludina occidentalis* in an unnamed pond in Lijiang.

Dalipaludina oxytropoides kept in ZMB are from Wiese et al. (2022). The specimens were photographed in a consistent orientation using a Sony alpha 7R 4 camera. Radulae and embryonic shells were extracted through dissection; radulae were cleaned by boiling in 1% NaOH solution for half an hour and rinsed with distilled water. Radulae and embryonic shells were coated with gold before scanning electron microscopy with a Zeiss *EVO LS10* scanning electronic microscope. The terms used for describing the operculum are according to Zhang and von Rintelen (2021).

Table 1. Shell measurements of *Dalipaludina* species. Values are arithmetic means (mm).

Species	H	W	W/H
Dalipaludina delavayana (n=25)	58.30 ± 8.30	38.30 ± 5.27	0.70 ± 0.04
Dalipaludina oxytropoides (n=91)	44.60 ± 5.78	32.30 ± 3.82	0.70 ±0.04
Dalipaludina occidentalis (n=27)	50.17 ± 4.17	41.00 ± 2.38	0.82 ±0.05
Dalipaludina pyramidella (n=30)	47.86 ± 7.06	41.34 ± 5.48	0.87 ± 0.05

DNA extraction and amplification

DNA was extracted from the foot tissue of eight individuals (10–20 mg of each individual), using a mollusc-specific CTAB/chloroform extraction protocol (Winnepenninckx et al. 1993). A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified through polymerase chain reaction (PCR) with the following primer pair: LCO1490, 5′-GGTCAACAAATCATAAAGATATTGG-3′ and COXB7R, 5′-ACCACCAGCTGGATCAAAAA-3′. (Schultheiß et al. 2011) PCR amplifications were conducted in 25 µl volumes under the following cycling conditions: initial denaturing step at 94 °C for 10 min, followed by 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, with a final extension step of 10 min at 72 °C. The purification and sequencing were conducted by Macrogen Europe, Amsterdam, Netherlands.

Phylogenetic analysis

Eight new DNA sequences (from eight individuals of two *Dalipaludina* species) have been uploaded to GenBank (accession numbers and museum voucher numbers, see Suppl. material 1). Additionally, three sequences of *Dalipaludina oxytropoides* published by Wiese et al. (2022) and sequences of 17 different genera of the subfamily Bellamyinae (Suppl. material 1) published by Stelbrink et al. (2020) and Hirano et al. (2019a) were downloaded from GenBank; *Viviparus viviparus* of the subfamily Viviparinae was selected as outgroup based on Stelbrink et al. (2020).

Sequences were aligned using MUSCLE as implemented in Geneious Prime 2020 (https://www.geneious.com). Genetic distances were calculated using MEGA X (Kumar et al. 2018). The dataset was tested in MEGA X for the best-fit model of sequence evolution by means of the Akaike and Bayesian information criteria; GTR+G+I was suggested as the best-fitting nucleotide substitution model. This model was employed in a maximum likelihood (ML) analysis conducted by RAxML as implemented in Geneious Prime 2020, with support estimated by 1,000 bootstrap replicates, and a Bayesian inference (BI) analysis performed with MrBayes 3.2.6 (Ronquist et al. 2012) as implemented in Geneious Prime 2020 with four independent chains for 5,000,000 generations, samplefreq = 1,000, burnin = 25%, and confirmed that convergence was reached based on the trace plots generated in Geneious Prime 2020.

Results

Genetic differentiation and phylogeny

COI p-distances between species of *Dalipaludina* are 1.81% to 3.98%; the p-distances between *Dalipaludina* and its closest relative, viz. the *Cipangopaludina*/*Margarya* complex, are 9.22%–14.98%, while p-distances within the *Cipangopaludina*/*Margarya* complex are 0–9.31%.

The phylogenetic trees reconstructed by BI and ML based on COI are highly congruent, therefore only the BI tree is shown (Fig. 2). The new genus *Dalipaludina* is monophyletic and distinct from all other included genera of Viviparidae. The three sequenced species of *Dalipaludina* are each recovered as monophyletic as well. *Dalipaludina occidentalis* is the sister species of *Dalipaludina delavayana*, and *Dalipaludina oxytropoides* is sister to both. *Dalipaludina* is the sister clade of a clade formed by the *Cipangopaludina*/*Margarya* complex, *Celetaia*, *Ussuripaludina*, *Hetergen*, *Torotaia*, *Anularya* and *Sinotaia*.

Systematics

Family Viviparidae J.E. Gray, 1847
Subfamily Bellamyinae Rohrbach, 1937

***Dalipaludina* Zhang, gen. nov.**
https://zoobank.org/6817CC4A-D98A-4D33-B890-49C91F64A1E5

Type species. *Paludina delavayana* Heude, 1889.

Etymology. "Dali" refers to the ancient Dali Kingdom (大理国) mostly situated in modern Yunnan, China; "paludina" refers the assignment to Viviparidae. The recommended Chinese name is "理田螺".

Diagnosis. Shell large, thin but solid; apex acute; teleoconch whorls with strong keel at suture, above suture relatively smooth or with several weak spiral threads or strong spiral cords, many thin and weak spiral threads on base; umbilicus narrow, sometimes bordered by a keel; exterior surface of operculum rather smooth, inner opercular region relatively small, nuclear region of operculum smooth, sometimes with small grains; outer marginal tooth with 9 to 11 small sharp cusps.

Comparative remarks. *Dalipaludina* gen. nov. resembles *Cipangopaludina* (widely distributed in East Asia), *Ussuripaludina* Zatravkin & Bogatov, 1987 (endemic to Far East), *Heterogen* Annandale, 1921 (endemic to Japan

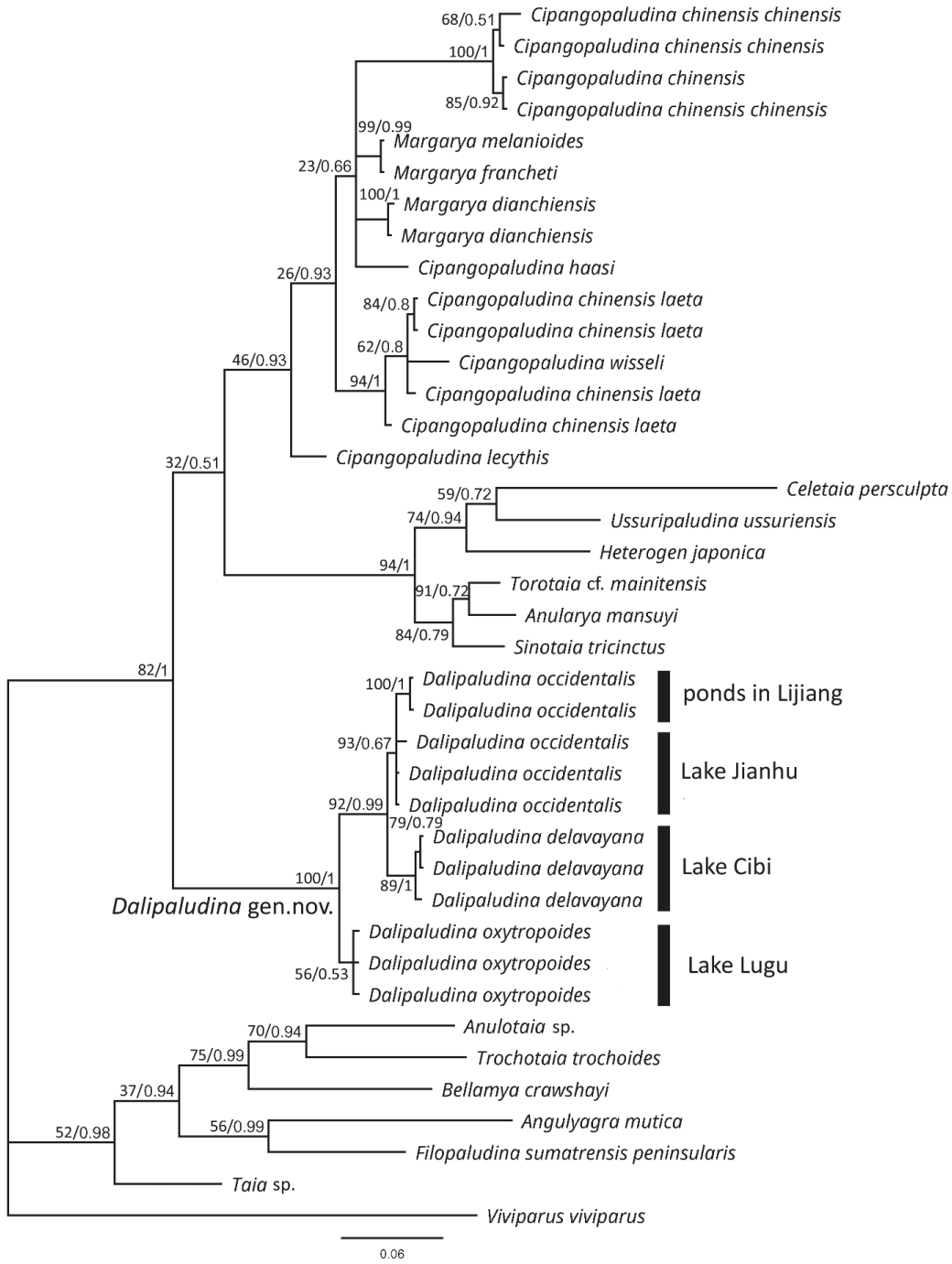


Figure 2. The BI tree based on cytochrome c oxidase subunit I (COI) showing the phylogenetic position of *Dalipaludina* gen. nov. within Viviparidae. Numbers above branches are ML bootstrap values/BI posterior probabilities.

and Korea), and *Torotaia* Haas, 1939 (endemic to Philippines, Sulawesi and New Guinea). It differs from *Cipangopaludina*, *Ussuripaludina*, *Heterogen* and *Torotaia* by having one strong keel at the suture, a shell base with many thin and weak spiral threads, a narrow umbilicus sometimes bordered by a keel, and operculum characters. The molecular phylogeny supports that *Dalipaludina* is distinct from *Cipangopaludina*, *Ussuripaludina*, *Heterogen* and *Torotaia*. The location of the testis in male *Dalipaludina* specimens in the mantle cavity supports the classification of this genus in the subfamily Bellamyinae. There are four known species of *Dalipaludina*.

Distribution. This genus is endemic to the lakes or ponds in northwest to northeast Yunnan, China.

***Dalipaludina delavayana* (Heude, 1889), comb. nov.**

Paludina delavayana Heude, 1889: 47 (“lacu Ta-li fou”, Lake Erhai).
Sinotaia delavayana – Qian et al. 2014.

Material examined. 2 ***syntypes***, IZCAS-FG-492553, IZCAS-FG-492554; 1 ***paratype***, MCZ-Mala-167342; 8 specimens in ZMB, collected by Le-Jia Zhang from around 0.5 to 1 metres depth in the north shore of Lake Cibi in Augest 2018, 2 specimens in ZMB, collected by Jiao-Wei Ning from around 1 to 2 metres depth in Lake Haixihai; 17 specimens in NJW, collected by Jiao-Wei Ning from around 1 to 2 metres depth in Lake Cibi.

Description. Shell (Fig. 3A–H) large, conical, thin, greenish yellow to dark olive in colour; up to six whorls at adulthood, including one relatively smooth protoconch whorl, apex acute, spire high; each teleoconch whorls with one strong keel at suture, above suture smooth or with many weak spiral threads, sometimes with additional two to three stronger cords, base of shell with many thin weak spiral threads, weak spiral cords and threads with short periostracal hairs; aperture ovate, less than half of shell in height, lip thin and simple, umbilicus narrow, sometimes bordered by a keel.

Operculum (Fig. 3I) corneous, ovate, rather thin, yellow, with reddish brown nuclear region, exterior surface of operculum rather smooth, inner opercular region relatively small, nuclear region smooth.

Radular (Fig. 7A, B) central tooth with one broad central denticle and with four small sharp cusps on either side; lateral tooth with one broad central denticle and four small sharp cusps on either side; inner marginal tooth with one broad central denticle and three small sharp cusps on either side; outer marginal tooth with 10 to 11 small sharp cusps.

Remarks. This species differs from the other *Dalipaludina* species by having a larger shell with a higher spiral. It may be difficult to distinguish from the smooth form of *Dalipaludina oxytropoides* from Lake Lugu based on morphology. However, this species is not recorded from Lake Lugu, and can be distinguished from *D. oxytropoides* based on differences in COI gene sequences. The recommended Chinese name of this species is “德拉维理田螺”.

Habitat and distribution. shallow water area with sandy and muddy bottoms in Erhai, Cibi and Haixihai, Yunnan, China.

***Dalipaludina oxytropoides* (Heude, 1889), comb. nov.**

Paludina oxytropoides Heude, 1889: 176 (‘lacu prope Tchao-tong’ = lake near Zhaotong City,Yunnan Province, China).
Vivipara oxytropoides – Kobelt 1909: 128.
Margarya sp. – Du et al. 2012: 45.
Margarya oxytropoides – Zhang et al. 2015: 777; Wiese et al. 2022.

Material examined. 2 ***syntypes***, IZCAS-FG-492586, IZCAS-FG-492587; 1 ***paratype***, MCZ-Mala-167343; 91 specimens in ZMB, collected by Frank Riedel from around 1 to 6 metres depth in all over the Lake Lugu in September and October 2014; 5 specimens in ZMB, collected by Le-Jia Zhang from 1 metre depth in the east shore of Lake Lugu in April 2022.

Description. Shell (Fig. 4A–H) large, conical, thin, greenish yellow to dark brown in colour; up to six whorls at adulthood, including one relatively smooth protoconch whorl, apex acute; each teleoconch whorls with one strong keel at suture, above suture with many rather weak spiral threads, sometimes with additional two to three strong cords, base of shell with many thin weak spiral threads, weak spiral cords and threads with short periostracum setae; aperture ovate, less than half of shell in height, lip thin and simple, umbilicus narrow, sometimes bordered by a keel.

Operculum (Fig. 4I) corneous, ovate, thin to relatively thick, yellow to reddish brown, with darker coloured nuclear region, exterior surface of operculum rather smooth, inner opercular region relatively small, nuclear region smooth or with small grains.

Radular (Fig. 7C, D) central tooth with one broad central denticle and three to four small sharp cusps on either side; lateral tooth with one broad central denticle and three to four small sharp cusps on either side; inner marginal tooth with one broad central denticle and four small sharp cusps on either side; each outer marginal tooth with 10 to 11 small sharp cusps.

Remarks. This species has the most variable shell within *Dalipaludina*. The form with strong cords of this species can be easily distinguished from the other *Dalipaludina* species. It used to be considered as a species of *Margarya* with a distribution in Lake Dianchi as well (Zhang et al. 2015). However, the COI-based phylogeny showed that “*Margarya oxytropides*” from Dianchi is not a species of *Dalipaludina*, but indeed a member of the *Cipangopaludina*/*Margarya* complex. The syntypes of *Paludina oxytropoides* Heude, 1889 from Zhaotong in Northeast Yunnan show a strong keel at the suture, two strong keels above the suture, a relatively smooth shell base with many weak spiral keels, and a narrow umbilicus with a keel around it. It is distinct from the “*Margarya oxytropides*” from Lake Dianchi but quite similar to the strongly keeled form of *D. oxytropoides* from Lake Lugu. Meanwhile, the shell morphology of “*Margarya oxytropides*” from Lake Dianchi matches the description and figure of *Cipangopaludina dianchiensis* Zhang, 1990 endemic to Lake Dianchi (Zhang 1990). Therefore, instead of *D. oxytropoides*, “*Margarya oxytropides*” from Lake Dianchi is here recognised as *Margarya dianchiensis* (Zhang, 1990) comb. nov. *Paludina oxytropoides* Heude, 1889 is reclassified as a member of *Dalipaludina*. The recommended Chinese name of this species is “尖龙骨理田螺”.

Habitat and distribution. shallow to medium-depth water area with sandy bottoms in Lake Lugu, Yunnan/Sichuan, China; lakes in Zhaotong, Yunnan, China.

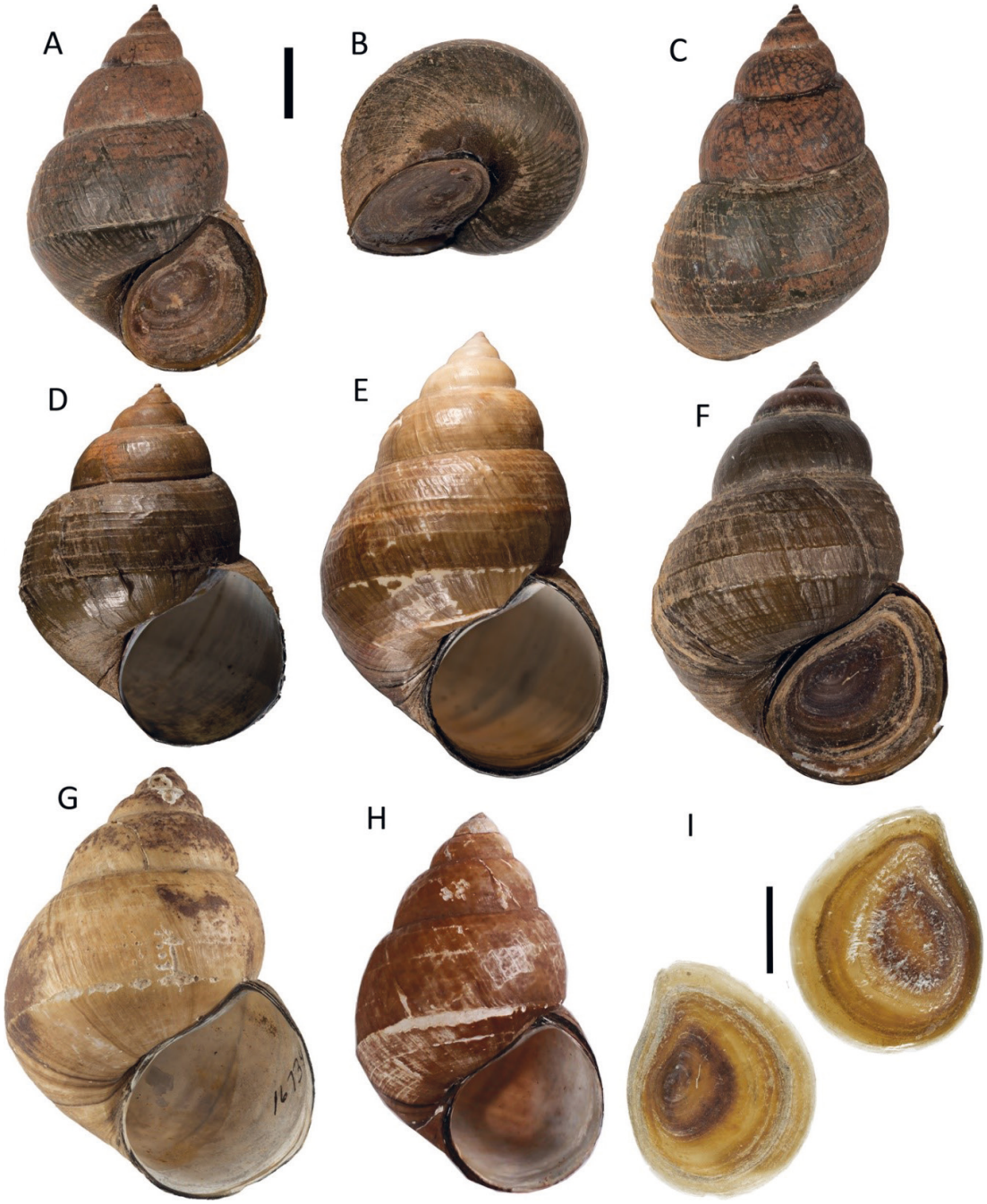


Figure 3. Shell and operculum of *Dalipaludina delavayana*. **A–C.** Specimen collected from Lake Cibi, ZMB Moll. 122707; **D, E.** Varieties collected from Lake Cibi, ZMB Moll. 122707; **F.** specimen collected from Lake Haixihai,LJZ; **G.** Paratype MCZ-Mala-167342, photo taken by Alana Rivera, Museum of Comparative Zoology, Harvard University, President and Fellows of Harvard College; **H.** Syntype IZCAS-FG-492554, photo taken by Kaibaryer Meng, Institute of Zoology, Chinese Academy of Sciences; **I.** Exterior (left) and interior (right) surface of operculum. Scale bar: 1 cm, **B–H** with the same scale bar of **A**.

***Dalipaludina occidentalis* (Annandale, 1924), comb. nov.**

Lecythoconcha malleata f. *occidentalis* Annandale, 1924: 415 ("Ho-ching and Shihku", Heqing County and Shigu town, Lijiang City, Yunnan, China).

Viviparus occidentalis – Yen 1942.

Material examined. 8 specimens in ZMB, collected by local fishermen from 3 to 5 metres depth in the south shore of Lake Jianhu. February 2020; 4 specimens in

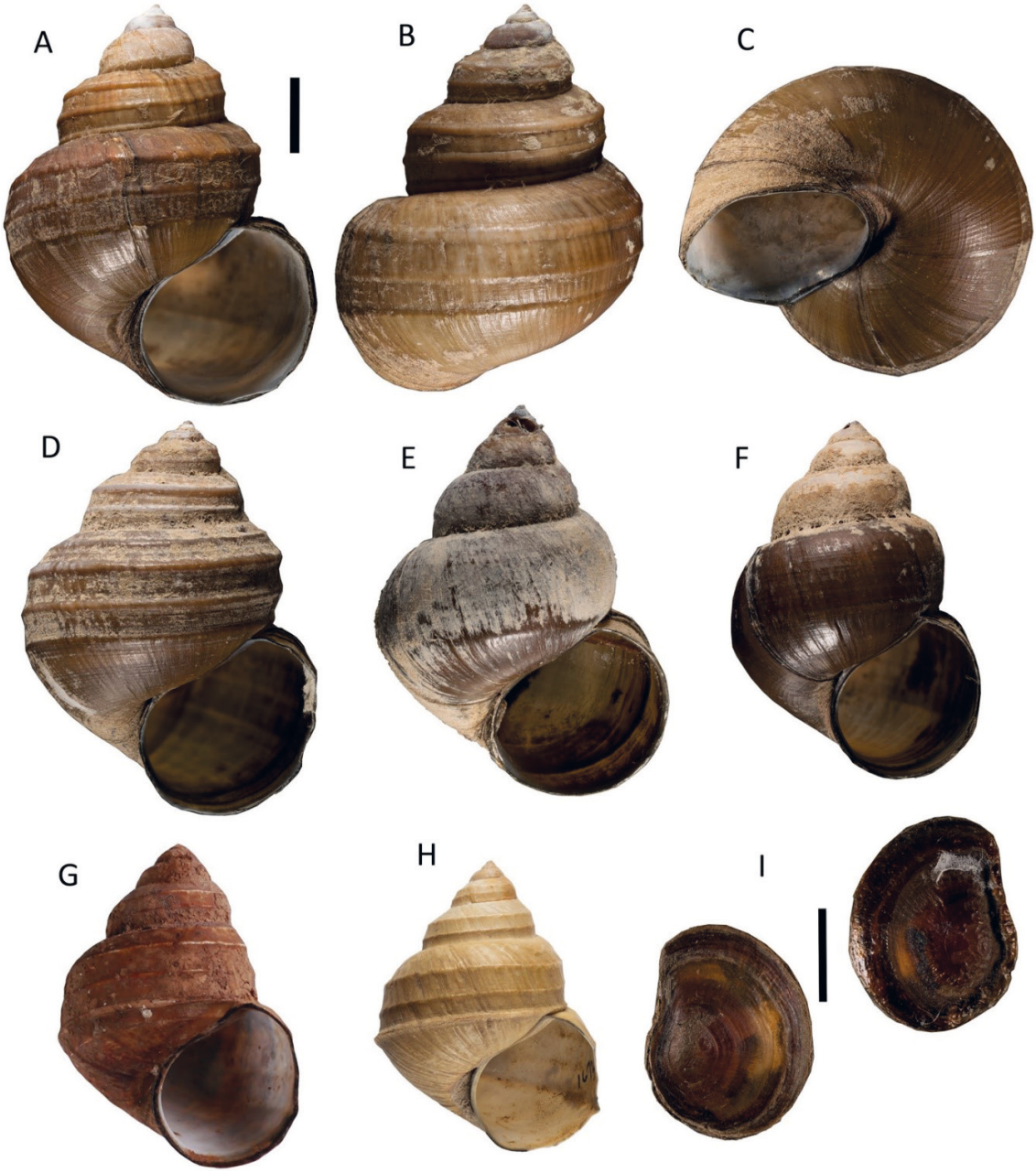


Figure 4. Shell and operculum of *Dalipaludina oxytropoides*. **A–C.** Specimen collected from Lake Lugu, ZMB.Moll. 250844; **D–F.** Varieties collected from Lake Lugu,ZLJ; **G.** Syntype IZCAS-FG-492587 from a lake in Zhaotong, Yunann, photo taken by Kaibaryer Meng, Institute of Zoology, Chinese Academy of Sciences; **H.** Paratype MCZ-Mala-167343 from a lake in Zhaotong, Yunann, photo taken by Alana Rivera, Museum of Comparative Zoology, Harvard University, President and Fellows of Harvard College; **I.** Exterior (left) and interior (right) surface of operculum. Scale bar: 1 cm, **B–F, H–I** with the same scale bar of **A**.

ZMB, collected by Le-Jia Zhang from 0.5 metres depth in an unnamed pond on the Sheshan hill in Lijiang City in April 2022; 19 specimens in NJW, collected by Jiao-Wei Ning from Lake Jianhu.

Description. Shell (Fig. 5A–G) large, broadly conical or sub globose, thin, olive to dark brown in colour; up to six whorls at adulthood, including one relatively smooth protoconch whorl, apex acute; teleoconch whorls inflated, occasionally shouldered, with one strong keel at suture, above suture with many weak spiral threads, sometimes with additional two to three stronger cords, base of shell with many thin weak spiral threads, weak spiral threads and cords with short periostracum setae; aperture ovate, slightly less than half of shell in height,

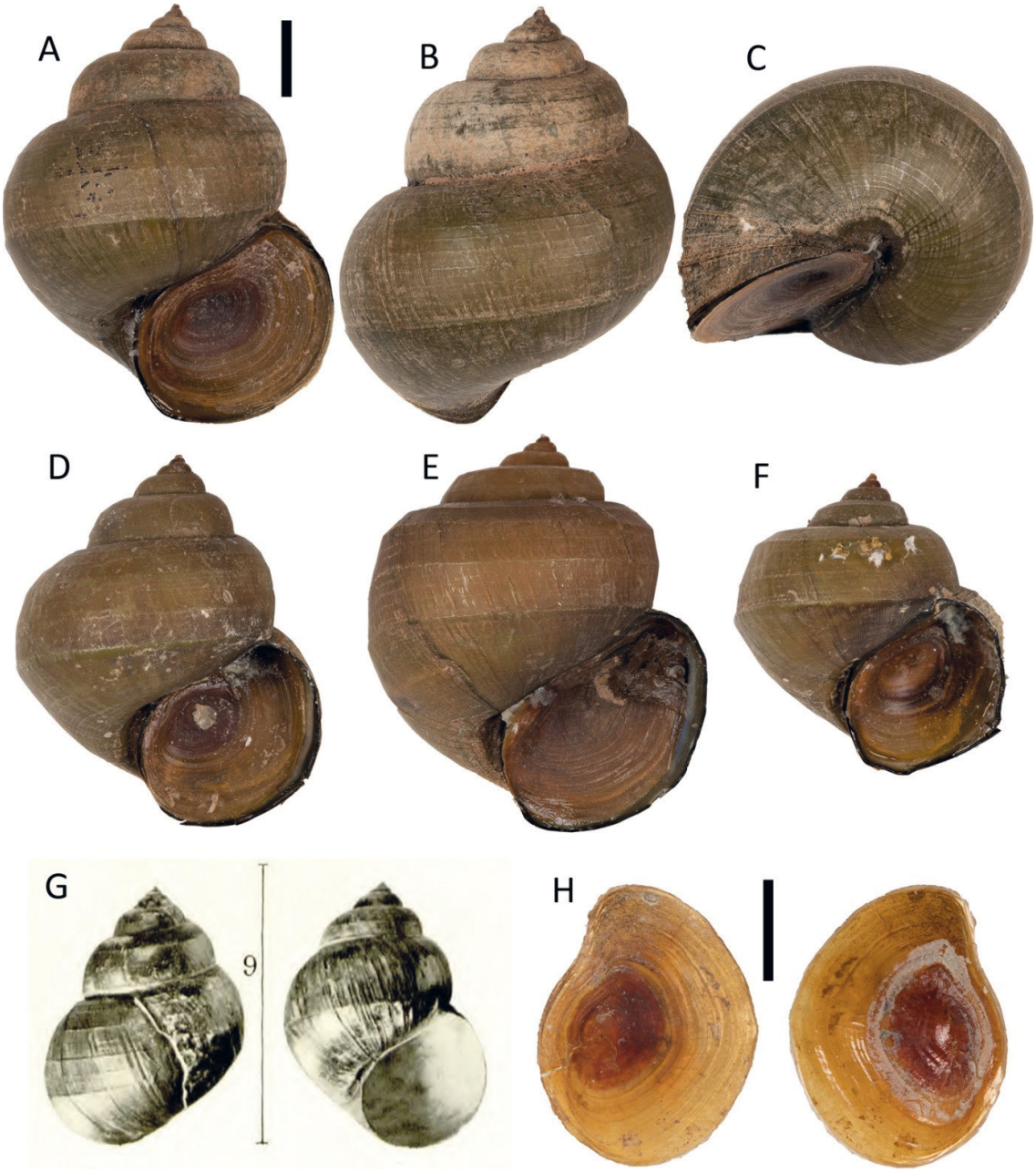


Figure 5. Shell and operculum of *Dalipaludina occidentalis*. **A–C.** Specimen collected from Lake Jianhu, ZMB.Moll. 122712; **D, E.** Varieties collected from Lake Jianhu, ZMB.Moll. 122713; **F.** Sub-adult collected from Lake Jianhu, ZMB.Moll. 122713; **G.** Original figure of the type specimen in Annandale, 1922; **H.** Exterior (left) and interior (right) surface of operculum. Scale bar: 1 cm, **B–F** with the same scale bar of **A**.

lip thin and simple, umbilicus narrow, sometimes bordered by akeel.

Operculum (Fig. 5H) corneous, ovate, rather thin, yellow, with red nuclear region, exterior surface of operculum rather smooth, inner opercular region relatively small, nuclear region smooth.

Radular (Fig. 7E, F) central tooth with one broad central denticle and four small sharp cusps on either side; lateral tooth with one broad central denticle and four small sharp cusps on either side; inner marginal tooth with one broad central denticle and four small sharp cusps on either side; outer marginal tooth with 9 to 10 small sharp cusps.

Remarks. This species differs from the other species of *Dalipaludina* by having rather inflated, shouldered whorls. Annandale (1924) presented a photo of the type specimen. The shell morphology and COI phylogeny of the speci-

mens collected from an unnamed pond in Lijiang City, which is very close to the type locality, confirm that this species should be assigned to *Dalipaludina*. The recommended Chinese name of this species is "滇西理田螺".

Habitat and distribution. Shallow ponds with muddy bottoms and abundant aquatic plants in Lijiang City, Yunnan, China; Shallow to medium-depth water area with muddy bottoms in Lake Jianhu, Jianchuan County, Yunnan, China.

Dalipaludina pyramidella (Du, Yang & Chen, 2011), comb. nov.

Trochotaia pyramidella Du, Yang & Chen, 2011: 85–89 (Yousuo village of Er-Yuan County, Yunnan, China).

Material examined. ***Holotype***, KIZ-DLN20100035; 19 ***paratypes***, KIZ-DLN20100036 to KIZ-DLN20100054; 3 specimens in ZMB, 7 specimens in NJW, collected by Jiao-Wei Ning from 1 metre depth in the ponds of Yousuo Village, Eryuan County.

Description. Shell (Fig. 6A–D) large, conical, thin, greenish brown to brown in colour; up to six whorls at adulthood, including one relatively smooth protoconch whorl, apex acute, spire low; each teleoconch whorls with one strong keel at suture, above suture smooth, with two rings of short periostracum setae, base of shell smooth, with many rather thin weak spiral threads; aperture ovate, almost half of shell in height, lip thin and simple, umbilicus narrow, sometimes bordered by a keel.

Operculum (Fig. 6E) corneous, ovate, rather thin, yellow or orange, with red nuclear region, exterior surface of operculum rather smooth, inner opercular region relatively small, nuclear region smooth.

Radular (according to Du et al. 2011) central tooth with one broad central denticle and three to four small sharp cusps on either side; lateral tooth with one broad central denticle and four small sharp cusps on either; inner marginal tooth with one broad central denticle and two small sharp cusps on either side; outer marginal tooth with 9 small sharp cusps.

Remarks. This species differs from the other species of *Dalipaludina* by having a relatively smooth shell with lower spire. Du et al. (2011) assigned this species to *Trochotaia* mainly based on the strongly keeled shell with lower spire. However, characters of the operculum, embryonic shells, the reduced number of cusps on the outer marginal teeth (9 in *D. pyramidella* vs 14–16 in *Trochotaia trochoides*) and the distribution in Yunnan strongly support its assignment to *Dalipaludina*. Species of *Trochotaia* have a very different operculum according to Zhang and von Rintelen (2021). This species is also quite similar to *Cipangopaludina miyagii* Kuroda, 1941 endemic to Kaohsiung, S Taiwan, China. It can be distinguished from *C. miyagii* by having a thinner shell, a shell base with many rather thin weak spiral threads, and a narrowly open umbilicus with a keel around it. The recommended Chinese name of this species is "塔形理田螺".

Habitat and distribution. shallow ponds with muddy bottoms and aquatic plant *Ottelia acuminata* (Gagnep.) in Yousuo Village, Eryuan County, Yunnan, China.

Discussion

The molecular and morphological data strongly support that four endemic viviparid species from Yunnan, formerly classified as species of *Cipangopaludina*, *Sinotaia*, *Margarya* or *Trochotaia*, should be assigned to a new genus described here: *Dalipaludina*. The former genus-level classification of these species was primarily based on shell morphology (Du et al. 2011; Qian et al. 2014). However, the shell morphology of freshwater snails can be quite similar due to convergence even between different genera. One remarkable example is the case of three highly sculptured but phylogenetically distinct viviparid genera (*Margarya*, *Tchangmargarya* and *Anularya*) endemic to the plateau lakes of Yunnan (Zhang et al. 2015). Our study has confirmed once again the importance of using an integrative approach in generic classification. In additional to shell characters, the operculum and radula, especially the cusps on the outer marginal teeth, provide important data for genus-level taxonomy of Viviparidae as well. The species-level phylogeny solely based on COI has been considered problematic in Viviparidae (Hirano et al. 2019b). However, at the genus level, it does not seem to be a big problem here (Fig. 2). Our BI tree based on partial COI sequences is broadly consistent with the genus-level topology for the Bellamyinae derived from an analysis of multiple genes in Stelbrink et al. (2020), suggesting that *Dalipaludina* likely falls into clade A of Bellamyinae proposed by Stelbrink et al. (2020).

Four species of *Dalipaludina* can be differentiated based on shell morphology. The scatter plot (Fig. 8) shows that *D. delavayana* has a larger and taller shell; *D. pyramidella* and *D. occidentalis* both have a lower spire, and *D. pyramidella* can be distinguished from *D. occidentalis* based on its less inflated whorls and comparatively smooth shell surface. The COI phylogeny has supported the reciprocal monophyly of the three sequenced species. The populations of *D. occidentalis* from two localities roughly 50 km apart cluster togther in the tree, without obvious geographic structuring (Fig. 2). The keeled, shouldered form of *D. occidentalis* (Fig. 5E) cannot be differentiated from the smooth form based on p-distance or phylogeny of COI as well. Moreover, the four species of *Dalipaludina* have allopatric distributions (Fig. 1B), and we have yet to find any lake or pond with more than one *Dalipaludina* species. Based on these results derived from morphological, molecular, and distribution data, we believe that there are four valid species of *Dalipaludina*.

Intraspecific diversity of shell morphology varies among the four species. *Dalipaludina delavayana* and *D. pyramidella* both display a relatively low intraspecific diversity; morphological diversity within *D. occidentalis* is higher; and that of *D. oxytropoides* is by far the

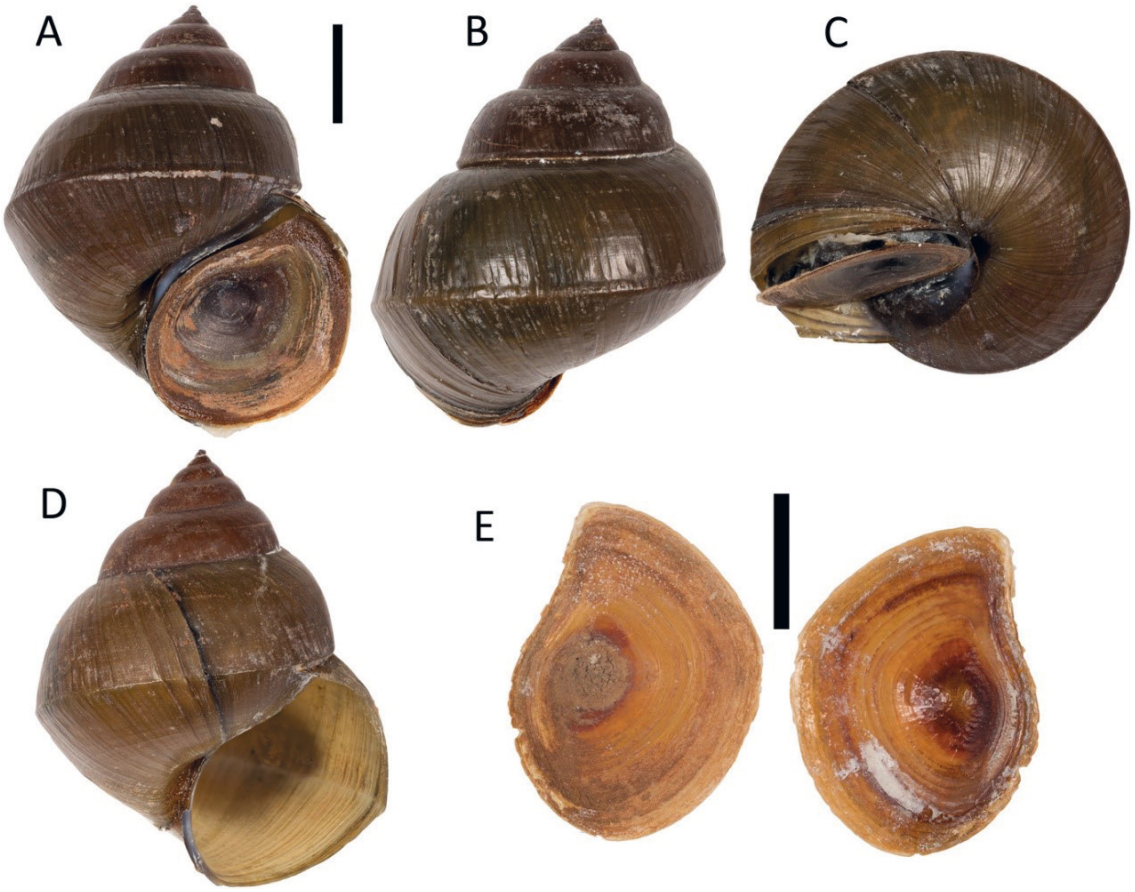


Figure 6. Shell and operculum of *Dalipaludina pyramidella.* **A–C.** Specimen collected from Eryuan County, ZMB.Moll. 122714; **D.** Varieties collected from Eryuan County, ZMB Moll. 122714; **E.** Exterior (left) and interior (right) surface of operculum. Scale bar: 1 cm, **B–D** with the same scale bar of **A**.

highest within the genus. An integrative study combining morphometrics and a COI phylogeny of *D. oxytropoides* from Lake Lugu ("*Margarya oxytropoides*" in Wiese et al. 2022) showed that it should be considered a single, but highly variable species. We have also observed many intermediate forms in this species.

In contrast to the other three endemic genera of Viviparidae from Yunnan (*Margarya*, *Tchangmargarya* and *Anularya*) mostly found in medium-depth to deep water (Zhang et al. 2015), *Dalipaludina* displays a preference for shallow water. In addition to large lakes like Lake Lugu or Jianhu, *Dalipaludina* also can be found in small shallow ponds, such as the artificial ponds for farming the aquatic vegetable *Ottelia acuminata* in Eryuan County (*D. pyramidella*) and an unnamed small pond on the top of Sheshan Hill in Lijiang (*D. occidentalis*). *D. oxytropoides* and *D. delavayana* are also more common in shallow water areas than in deeper water. However, *Dalipaludina* is only found in lentic environments. It has never been found in rivers or any other lotic environment.

The lowest altitude where we have found *Dalipaludina* species is in Eryuan County, around 1974 m a.s.l., and one historical record of *D. oxytropoides* in Zhaotong County from ~1920 m a.s.l. (Heude 1886); Lake Lugu harbouring *D. oxytropoides* is ~2698 m a.s.l., which is the highest altitude record of Viviparidae (Fig. 1B). Among the other three viviparid genera endemic to Yunnan Plateau, only *Margarya* is found within a similar but smaller altitudinal range (1886–2192 m a.s.l), while *Tchangmargarya* (1770–1907 m a.s.l.) and *Anularya* (1284–1721 m a.s.l.) inhabit a relatively lower altitude with no overlap with that of *Dalipaludina*. Viviparids from elsewhere in the world occur at much lower altitudes. In conclusion, *Dalipaludina* displays an adaptation to the shallow lentic environment in high altitude regions, and occurs at the highest altitude of any viviparid.

With the discovery of the new genus *Dalipaludina*, the Yunnan Plateau now harbours four endemic genera of Viviparidae, confirming again that it is the most important diversity hotspot of Viviparidae in the world. However, almost all of these species are threatened by human activities such as overharvesting, pollution, and habitat destruction (Yang et al. 2004; Zhang et al. 2015). *D. pyramidella* nearly disappeared from its only known site, the ponds in Eryuan County, during our recent surveys in 2021; the present population of *D. oxytropoides*

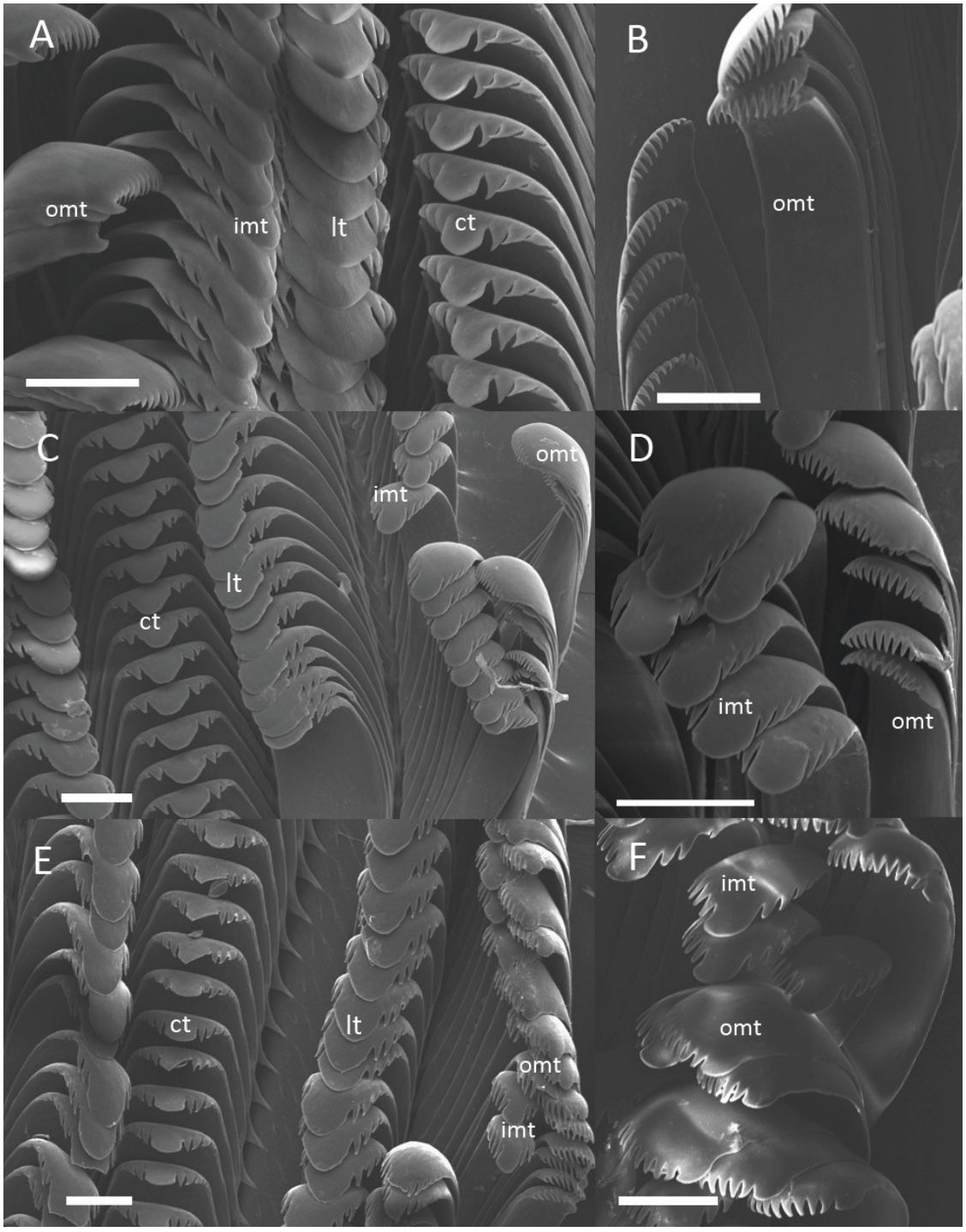


Figure 7. SEM photo of the radula and the protoconch of *Dalipaludina* species. **A, B.** Radula of *Dalipaludina delavayana*, A-ZMB. Moll. 181719, B-ZMB.Moll. 122707; **C, D.** Radula of *Dalipaludina oxytropoides*, ZMB.Moll. 250844; **E, F.** Radula of *Dalipaludina occidentalis*, ZMB.Moll. 122713. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth. Scale bars: 100 μm.

in Lake Lugu has declined dramatically compared to that in the 1990s according to local fishermen. Most endemic viviparid species of *Margarya*, *Tchangmargarya* and *Anulary*a are on the national Red List of China (Wang and Xie 2005), and one species *Margarya melanioides* Nevill, 1877 is a Class 2 species under state protection in the

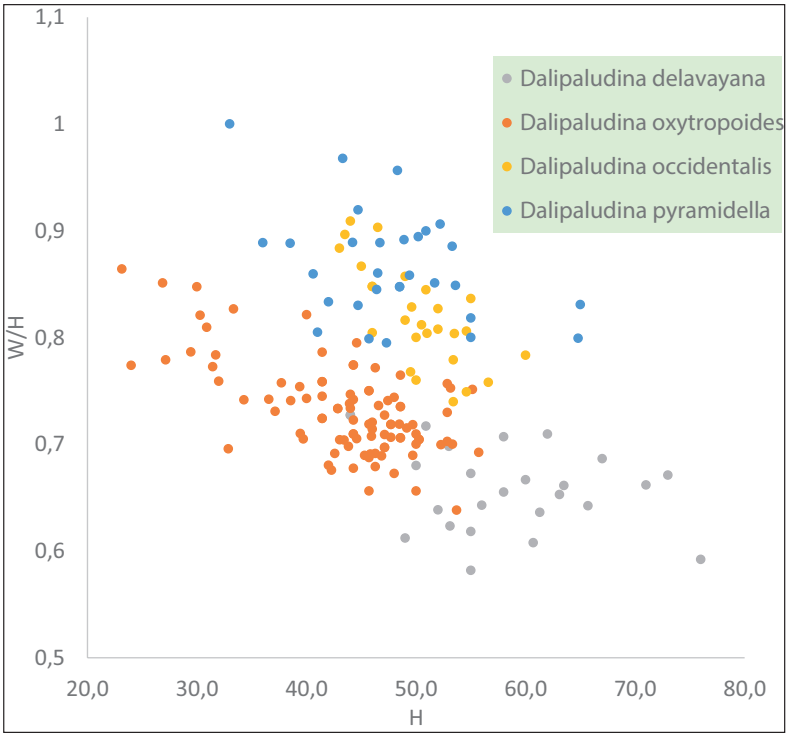


Figure 8. Comparison of *Dalipaludina* species with respect to parameters H (in mm) and W/H.

latest List of State Key Protected Wild Animals of China in 2021. Since all four *Dalipaludina* species are restricted to just one or two lakes or several small ponds, specific conservation measures should be applied urgently for each species based on more detailed population biology and ecology studies. The phylogeny based on genomic data, such as data from exon capture method, can also improve our understanding of the evolution of these rare species endemic to the plateau lentic environment.

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Supplementary material 1

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An extinct species of *Tchangmargarya* (Gastropoda: Viviparidae) from quaternary lacustrine deposits of a group of vanished lakes in Yunnan, China

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ABSTRACT

An extinct freshwater molluscan fauna has been discovered from Quaternary lacustrine deposits of a recently vanished group of lakes and surrounding lacustrine deposits in the Luliang Basin, Yunnan Province, China. Three species of *Tchangmargarya* He, 2013 are dominant and distributed sympatrically here. One of them is new to science. Here we describe the three *Tchangmargarya* species from the Luliang Basin, including the new species, *Tchangmargarya cuan* n. sp., and compare the species composition of this fauna with the other extant lacustrine mollusc fauna of Yunnan. The vanished lakes group in Luliang is the first case of sympatric distribution within *Tchangmargarya*. It is considered to have once been a diversity hotspot of *Tchangmargarya* and represents a vanished Quaternary lacustrine habitat in Yunnan.

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Quaternary; lacustrine; sympatry; viviparid snails

Introduction

Tchangmargarya He, 2013 is a genus of large, ornamented viviparid snails endemic to several lakes located to the east of the Liangwang Mountains, Yunnan, China (Zhang 2017). It can be distinguished from the other endemic viviparid genera of Yunnan, *Margarya* Nevill, 1877 and *Anularya* Zhang et al. 2015, based on the sculpture of shell, operculum with an enamel region that is thickened near the inner opercular region, and anatomical characters, especially the reddish-yellow prostate and much shorter cephalic tentacles in males, and its distinct placement in molecular phylogenies (Zhang et al. 2015; Zhang and von Rintelen 2021). There are three known species in this genus: *Tchangmargarya yangtsunghaiensis* Tchang & Tsi, 1949, *Tchangmargarya multilabiata* Zhang et al. 2015, and *Tchangmargarya ziyi* Zhang, 2017. *T. ziyi* may have recently disappeared from the studied area due to habitat destruction (Zhang 2017).

The Luliang Basin is a large basin east of the Liangwang Mountains. There were more than a dozen lakes in this basin, including the Luliang Lake (also known as Zhongyuanze or Dahaizi), Xiaohaizi Lake, and Baishuitang Lake. All these lakes were very close to each other and intermittently connected by the Nanpan River during flood seasons. The largest lake, Luliang Lake, had an area of 18.25 km^2 and an average depth of 2.5 metres in 1949 (Ji 1989). It was probably much larger during the Qing Dynasty (1636–1912; Ji 1989). However, all of these lakes were drained artificially during the 1960s and 1970s to prevent flood disasters and allow the cultivation of farmland (Ji 1989). Only one degenerated shallow pond, Baishuitang, still exists today. No biodiversity survey has yet been conducted in these lakes and nearby regions.

A large number of ornamented viviparid fossils has been reported from the late Pliocene Ciying Formation in Qujing City, which is located to the north of the Luliang Basin (Yen 1935; Pan 1986). The extant ornamented viviparid species *T. multilabiata* is distributed in the lakes east of the Luliang Basin (Zhang 2017). However, no fossil or extant mollusc species have been reported from the Luliang Basin. During several recent investigations in the Luliang Basin, we have discovered many well-preserved freshwater mollusc shells in Quaternary lacustrine deposits and the dry lakebed of this area. Among these shells, we have identified three dominant ornamented viviparid species. The present comparative morphological study demonstrates that all of them are species of *Tchangmargarya*. One of these species is considered to be undescribed. In this paper, we describe the three *Tchangmargarya* species from the Luliang Basin and compare the freshwater mollusc fauna of this region with that of other extant lacustrine habitats in Yunnan.

Material and methods

Altogether nine field works were conducted in four areas (type locality, and areas A, B and C) of the

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Luliang Basin between January 2019 and October 2022 (Figure 1). We could not find any naturally exposed fossil-bearing interval. All the specimens studied here were collected in the soil or mudstone by the roadside in the backyard of Wanghai Temple, or material excavated from the nearby recent building foundations and the canal renovation in areas A, B and C. The dry lakebed of Luliang Lake was surveyed as well (Figure 1C). The height (H) and width (W) of shells were measured with a caliper to a precision of 0.1 mm. Number of whorls (N) was counted to a precision of 0.5 whorls. A scatter plot was drawn with respect to parameters H and W/H using Excel 2016. Only mature and complete specimens (total 91 specimens) were included in the shell measurements. The newly collected specimens were compared to the specimens of *Tchangmargarya* species studied in Zhang et al. (2015) and Zhang (2017). Specimens were photographed in a consistent orientation using a Sony Alpha 7R IV camera. Type specimens are deposited in the Kunming Institute of Zoology (KIZ), and the private collections of Le-Jia Zhang (ZLJ) and Xiao-Dong Wang (WXD).

Systematics

Viviparidae Gray, 1847

***Tchangmargarya* He**, 2013

Type species: *Margarya yangtsunghaiensis* Tchang & Tsi, 1949, by original designation

***Tchangmargarya cuan* n. sp. https://zoobank.org/28C7D3CD-70B3-4DD4-998A-EEB4A922A456**

Figure 2

Holotype. KIZ23061, collected from the backyard of Wanghai Temple, buried in soil, Luliang County, Yunnan, China.

Paratypes. 37, 6 collected from the type locality, 2 collected from area A, 21 collected from area B, 7

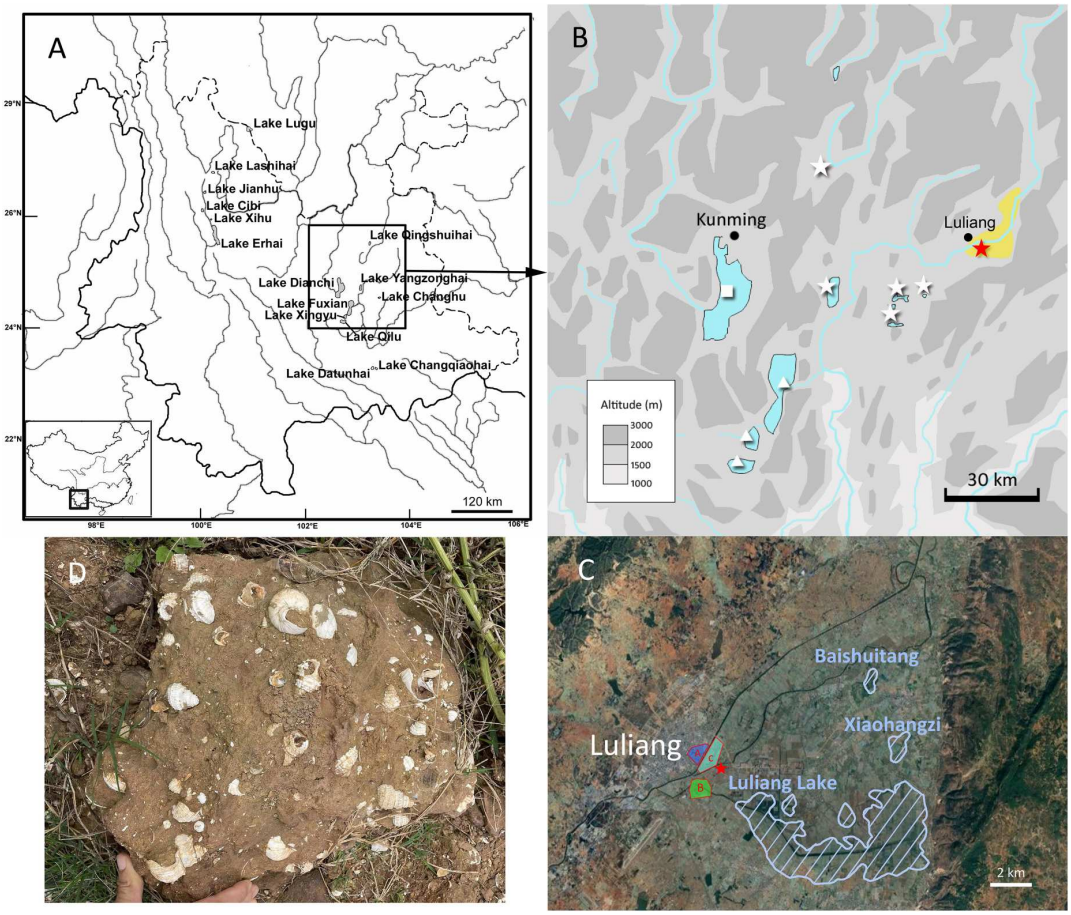


Figure 1. Location map and the collecting sites in Luliang Basin, Yunnan, China. **A,** Map of Yunnan Province (adapted from Zhang et al. 2015); **B,** map of Central East Yunnan showing the altitude (adapted from Zhang 2017), star – *Tchangmargrya* spp. from Luliang, white star – *Tchangmargrya* spp., square – *Margarya* spp., triangle – *Anularya* spp., shaded area – Quaternary basin in Luliang (adapted from Ma 2002); **C,** type locality (star) and the three other collecting areas (A, B, C) in Luliang, the areas of the lakes in 1949 is according to Army Map Service (1954); **D,** a piece of mudstone containing the shells of three *Tchangmargrya* species in situ.



Figure 2. Shells of *Tchangmargarya cuan* n. sp. from Luliang County. **A–D,** Holotype; KIZ; **E–G,** paratypes showing varieties; **H–J,** sub-adult individual; **K–P,** juvenile individuals. Scale bar = 1 cm.

collected from area C (Figure 1C); 20 kept in KIZ (KIZ23062-KIZ23081), 6 kept in ZLJ and 11 kept in WXD.

Material examined

Type material. See above.

Diagnosis

Shell large and thick, subglobose, the last two whorls with large sharp spines on the shoulders and very weak keels or even no keels, inner lips from different growing periods outward-folding and tightly overlapping.

Description

Shell large, subglobose, very thick, solid and heavy; mostly with 5.5–6.5 whorls, including two protoconch whorls, teleoconch whorl inflated, with a well-developed ramp-like shoulder; third and fourth whorls with quite extended smooth shoulders, two to three weak spiral ridges very close to each other located between the two strong keels, many axial ribs forming nodules at the intersection of spiral keels and axial ribs; last two whorls with 10–15 large sharp spines on the shoulder, three to four really weak spiral keels, axial ribs almost disappeared; base of shell smooth or with one weak keel around umbilical area; aperture ovate, inner lips from different growing periods outward-folding and tightly overlapping, forming a crescent-moon-shaped callus-like structure completely covering umbilicus.

Juvenile shell large, with four whorls; first two protoconch whorls with several smooth spiral keels; third and fourth whorls with spiral keels and axial ribs, forming nodules at the intersection of spiral keels and axial ribs; base of shell with many obvious axial ribs; aperture ovate, inner lip outward folding, completely covering umbilicus.

Remarks

This species is most similar to *T. multilabiata*, which has a crescent moon-shaped callus-like structure covering the umbilicus. The adult shell of *T. cuan* n. sp. can be

Table 1. Shell measurements of *Tchangmargarya* species. Values are arithmetic mean (mm) followed by standard deviation except for number of whorls. For abbreviations see Material and Methods section; *n* = number of sampled specimens.

Species	*n*	Locality	H	W	N	W/H
T. cuan n. sp.	38	Luliang Basin	56.62 ± 8.25	46.80 ± 7.66	5.5–6.5	0.83 ± 0.06
T. multilabiata	33	Luliang Basin	46.21 ± 6.07	33.58 ± 4.26	5.5–6.5	0.73 ± 0.07
T. ziyi	20	Luliang Basin	40.08 ± 3.33	25.09 ± 1.75	6–7	0.63 ± 0.04
T. multilabiata	59	Lakes in Shilin	44.38 ± 8.11	32.01 ± 5.28	6–7	0.73 ± 0.03
T. ziyi	50	Lake Babuhai	42.54 ± 3.85	27.84 ± 2.20	6–7.5	0.66 ± 0.02
T. ziyi (subfossil)	17	Lake Babuhai	55.38 ± 5.00	29.9 ± 2.64	6–8.5	0.54 ± 0.05
T. yangtsunghaiensis	28	Lake Yangzonghai	48.29 ± 6.69	29.95 ± 3.78	6–7	0.62 ± 0.03

easily distinguished from that of *T. multilabiata* based on the larger, more subglobose shell with the same number of whorls (Table 1; Figure 4), the last two whorls with large sharp spines on the shoulders and very weak spiral ridges or even no spiral ridges. The juvenile shell of *T. cuan* n. sp. has more extended shoulders and two to three weak keels on whorls; meanwhile the juvenile shell of *T. multilabiata* has less extended shoulders and a total of three to four keels with similar thickness.

Etymology: 'cuan' refers to the lost ancient Cuan kingdom from the type locality of this species, Luliang, Yunnnan. Formed as a noun in apposition. The recommended Chinese name is '爨乡玺螺蛳'.

Distribution and ecology

This species is only found in lacustrine deposits near the downtown area of Luliang County. Only white shells without periostracum have been found. This species is found together with the shells of the freshwater snails *T. multilabiata*, *T. ziyi*, at least four species of *Paraprososthenia* Annandale, 1919, *Fenouilia* sp., *Lacunopsis* sp., some unidentified species of Pomatiopsidae Simpson, 1885, as well as the freshwater mussels *Rhombuniopsis* sp. and *Sinanodonta* sp.

Tchangmargarya multilabiata Zhang et al. 2015
Figure 3A–D

Tchangmargarya multilabiata Zhang et al. 2015: 785–787 (Changhu Lake, Shilin County, Yunnan Province, China).

Material examined

Other material. Total of 33 specimens collected from Luliang County, 3 collected from the type locality of *Tchangmargarya cuan* n. sp., 1 collected from area A, 26 collected from area B and 3 collected from area C (Figure 1C); 10 kept in KIZ (KIZ23082- KIZ23091), 3 kept in ZLJ and 20 kept in WXD.

Description

Shell large, conical to subglobose, thick, solid; mostly with 5.5–6.5 whorls, including two protoconch whorls, teleoconch whorl inflated, with a well-developed ramp-like shoulder; teleoconch whorls with three strong spiral keels and many axial ribs, forming nodules at the intersection of spiral keels and axial ribs; base of shell with two weak incomplete spiral keels with weak nodules around umbilical area; aperture ovate, inner lips from different growing periods extremely outward-folding and tightly overlapping, forming a very large, thick crescent moon-shaped callus-like structure completely covering umbilicus, clearly visible from dorsal view of shell.

Remarks

Compared to the extant *T. multilabiata* from the lakes in Shilin County, the extinct population of *T. multilabiata* from Luliang Basin has a much larger crescent moon-shaped, callus-like structure and one keel less on each whorl. However, the general shape and size are quite similar (Table 1).

Distribution

Lacustrine deposits near Luliang County; lakes in Shilin County.

Tchangmargarya ziyi Zhang, 2017
Figure 3E

Tchangmargarya ziyi Zhang, 2017: 252–257 (Bank of Lake Babuhai in the JializeWetland, Yanglin Town, Songming County, Kunming City, Yunnan, China).

Material examined

Other material. Total of: 20 specimens collected from Luliang County, 4 collected from the type locality of *Tchangmargarya cuan* n. sp., 3 collected from area A, 11 collected from area B and 2 collected from area C (Figure 1C); 10 kept in KIZ (KIZ23092- KIZ23101), 4 kept in ZLJ and 6 kept in WXD.

Description

Shell large, conical, thick, solid; mostly with 6–7 whorls, including two protoconch whorls, each teleoconch whorl with a well-developed ramp-like shoulder; teleoconch whorls with four spiral keels and many axial ribs, forming nodules at the intersection of spiral keels and axial ribs, the second spiral keel usually weaker than

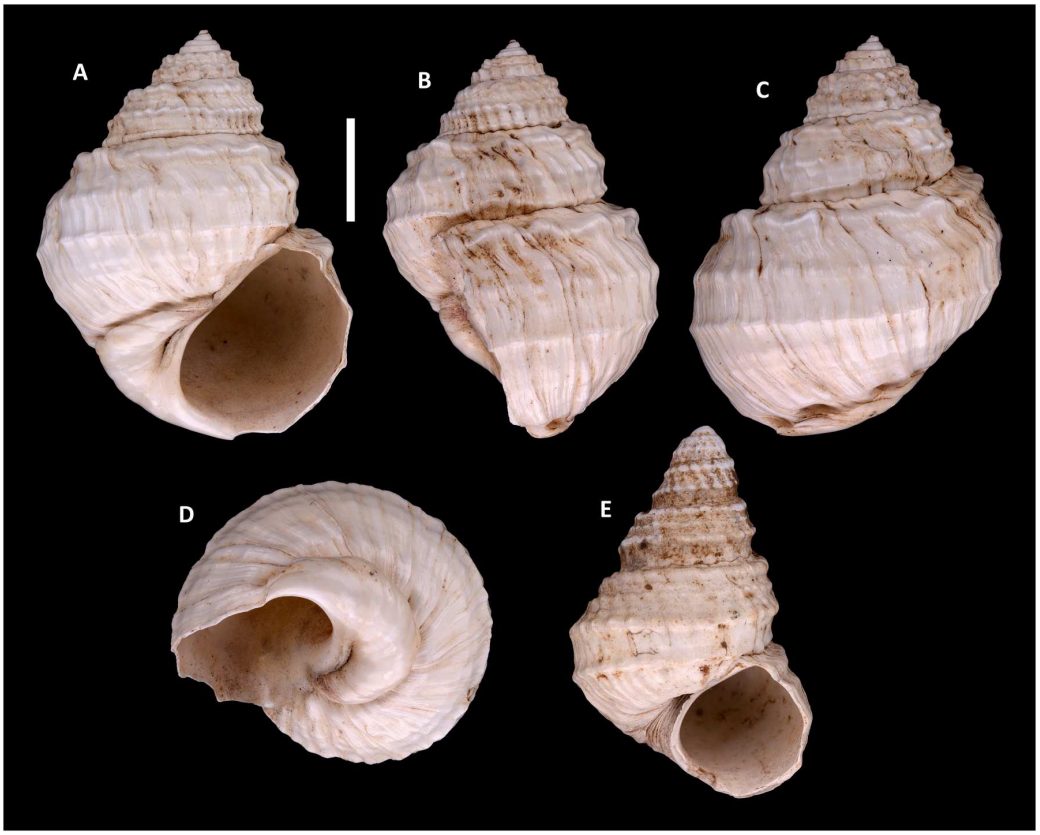


Figure 3. Shells of other *Tchangmargarya* species from Luliang County. **A–D,** *Tchangmargarya multilabiata*; **E,** *Tchangmargarya ziyi*. Scale bar = 1 cm.

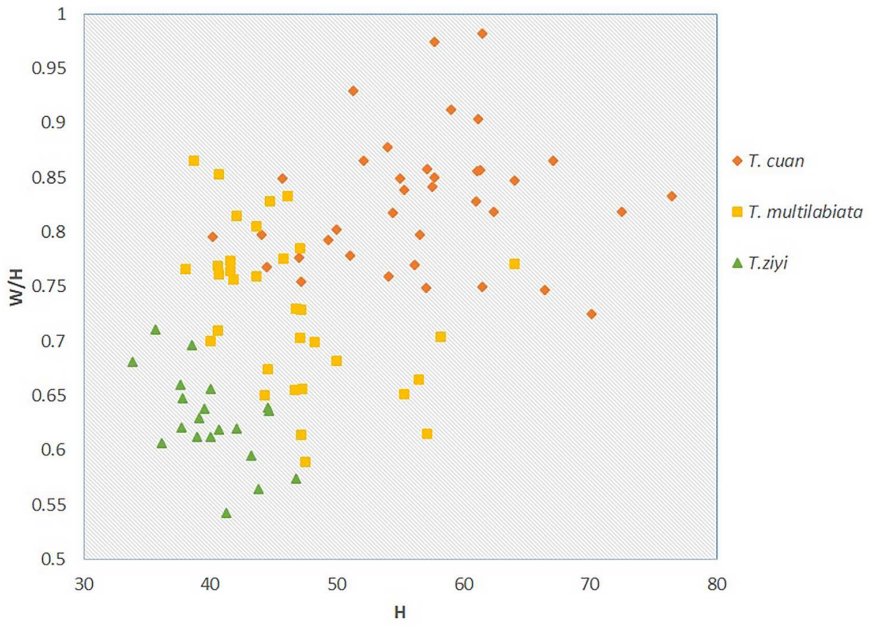


Figure 4. Comparison of the *Tchangmargarya* species from Luliang County with respect to parameters H and W/H.

the other three keels; base of shell with two weak incomplete spiral keels with weak nodules around umbilical area; aperture ovate, less than shell height, umbilicus covered by inner lip or slightly open.

Remarks

Except for the weaker second spiral keel, the extinct *T. ziyi* from Luliang Basin is almost identical to the recent *T. ziyi* from Lake Babuhai in shell morphology (Table 1).

Distribution

Lacustrine deposits near Luliang County; Lake Babuhai, Jialize Wetland, Yanglin, Songming County.

Discussion

The present study reveals that at least three species of *Tchangmargarya*, including one new species, were distributed sympatrically in the vanished lakes group of Luliang Basin. This is the first case of sympatric distribution found within *Tchangmargarya*. All other known distributions of *Tchangmargarya* species are strictly allopatric (Zhang et al. 2015). The vanished lakes group in Luliang Basin was arguably a diversity hotspot of *Tchangmargarya*. In addition, as also suggested by Zhang et al. (2015) and Zhang (2017), the Liangwang Mountains are again shown to represent a geographic barrier for the three endemic ornamented viviparid genera of Yunnan (Figure 1B).

Freshwater snails, especially Viviparidae, sometimes can display a high plasticity of shell morphology. *Dalipaludina oxytropoides* (Heude, 1889) endemic to Lugu Lake in Yunnan has a series of continuous variation from a smooth to a strongly-keeled shell (Wiese et al. 2022; Zhang et al. 2023). Another example is the case of *Heterogen longispira* (Smith, 1886) endemic to Biwa Lake, *Heterogen* sp. endemic to Chikubu Island of Biwa Lake and the widespread *Heterogen japonica* (Martens, 1861); the three species can be easily distinguished based on their shell morphology, especially by characters of the juveniles; however, they can hardly be differentiated either in a multi-locus phylogeny or using genome-wide SNPs (Hirano et al. 2019), which suggests the possibility that they rather represent ecotypes instead of species.

The populations of the three *Tchangmargarya* species that are no longer found in the Luliang Basin can only be studied by morphology. Unlike the case from Fuxian Lake, we did not find obvious intermediate variation among these three species. The scatter plot displayed a discontinuous difference of the three species (Figure 4). In addition, the two described species from the Luliang Basin (*T. multilabiata* and *T. ziyi*) are relatively morphologically conservative compared to the extant or recent populations of these two species from other localities. The individuals of *T. multilabiata* found together with *T. cuan* n. sp. differ from the extant individuals of *T. multilabiata* by the much larger crescent moon-shaped, callus-like structure; however, the combination of the other shell characters still supports them as being a variation of *T. multilabiata*. The individuals of *T. ziyi* found together with *T. cuan* n. sp. are more similar to extant individuals of *T. ziyi* instead of the subfossil ones, especially considering shell size and the number of keels (Table 1). Therefore, we consider the morphologically distinct *T. cuan* n. sp. to represent a biological species instead of a morphotype.

Sharp spines are relatively rare shell characters within Viviparidae. Excepting *Tchangmargarya*, they are found only in *Margarya melanioides* Nevill, 1877, *Margarya spinicostata* Li, 1987 †, *Margarya nanningensis* Ying, Fürsich & S. Schneider, 2013 †, *Macromargarya aliena* Ying, Fürsich & S. Schneider, 2013 †, *Taia* spp., and *Torotaia partelloi* (Bartsch, 1909). *Tchangmargarya cuan* n. sp. is the third viviparid species with sharp spines from Yunnan. It undoubtedly can be assigned to *Tchangmargarya* because of the sculpture of the juvenile shell. The unique crescent moon-shaped callus-like structure on the umbilicus area of this species indicates its close relationship to *T. multilabiata*. This character is not found in any other ornamented viviparid species from Yunnan. Despite having a similar number of whorls in the adult shell, *T. cuan* n. sp. is much larger than other species of *Tchangmargarya* (Table 1; Figure 4). The magnificent large shell and large sharp spines make it one of the most impressive ornamented viviparid species. All the specimens of *T. cuan* n. sp. were collected from the excavated soil and mudstone of construction works in a narrow region close to the old town centre of Luliang County, which is outside the area covered by the lakes in 1949 (Figure 1C). Indeed, the old town centre has been far from the lake region since 1408 or earlier (Ji 1989). However, the Quaternary lacustrine deposits do extend to the old town centre of Luliang, indicating that this area was part of the former lake (Figure 1B, Ma 2002). We did not find any specimens of this species from the dry lakebed of the former Luliang Lake, although some specimens of *T. multilabiata*, which are almost identical to extant individuals in Shilin County, were found there. The other freshwater molluscs in this assemblage, such as *Paraprososthenia* spp., *Fenouilia* sp., *Lacunopsis* sp., *Rhombuniopsis* sp. and *Sinanodonta* sp. are all modern endemic groups also found in the extant lakes of Yunnan, such as Erhai, Fuxian and Dianchi (Davis et al. 1983; Huang et al. 1986; Shi et al. 2020). The species composition, including multiple endemic species of ornamented viviparids within one genus and a large number of endemic small gastropods and freshwater mussels, makes the

group of vanished lakes in Luliang Basin quite similar to the extant species composition of the largest lake in Yunnan, Dianchi (Zhang et al. 1995). Therefore, although it still needs further study, such as radiocarbon dating, to ascertain the time of extinction, we suggest that the freshwater molluscs including *T. cuan* n. sp. from Luliang Basin should at least represent an extinct Quaternary lacustrine fauna.

The lacustrine environment of Yunnan harbours an extremely high biodiversity and endemism of freshwater snails. The shells of *Tchangmargarya* from the lost fauna of Luliang Basin reveal a formerly unknown vanished hotspot of freshwater molluscs in Yunnan. More surveys and studies of this area in the future will contribute to our better understanding of the biodiversity and evolutionary history of these endemic ornamented snails.

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Disclosure statement

No potential conflict of interest was reported by the authors).

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Chapter 4. Zhang LJ, Yen YH, Chen ZY, Du LN, Ng TH & von Rintelen T. A new genus of river snails, *Bakyietaia* (Mollusca, Viviparidae), from South China and mainland Southeast Asia. Manuscript.

A new genus of river snails, *Bakyietaia* (Mollusca, Viviparidae), from South China and mainland Southeast Asia

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ABSTRACT

A new genus of the freshwater snail family Viviparidae (river snails), ***Bakyietaia* gen. nov.**, is described based on a comprehensive study of its morphology, anatomy, mtDNA (COI), ecology and distribution. This genus is widely distributed in South China and Mainland Southeast Asia. It can be distinguished from all other viviparid genera, especially *Angulyagra*, *Sinotaia* and *Anulotaia*, by characters of the inner lip of the shell, operculum and marginal teeth of radula. Based on an integrative taxonomy approach, 18 species are assigned to this new genus and described here, including 7 new combinations and 11 new species: ***Bakyietaia subcostata* comb. nov.**, ***Bakyietaia polyzonata* comb. nov.**, ***Bakyietaia boettgeri* comb. nov.**, ***Bakyietaia duchieri* comb. nov.**, ***Bakyietaia guangdungensis* comb. nov.**, ***Bakyietaia mutica* comb. nov.**, ***Bakyietaia wilhelmi* comb. nov.**, ***Bakyietaia chenghuang* sp. nov.**, ***Bakyietaia jingweiae* sp. nov.**, ***Bakyietaia liusanjieae* sp. nov.**, ***Bakyietaia paradoxa* sp. nov.**,

***Bakyietaia avisvenatoris* sp. nov., *Bakyietaia liangzhuorum* sp. nov., *Bakyietaia luikongi* sp. nov., *Bakyietaia fontinalis* sp. nov., *Bakyietaia naiadica* sp. nov., *Bakyietaia luuemxlong* sp. nov.** and ***Bakyietaia indrapura* sp. nov.**. The monophyly of most morphospecies (17/18) is supported by the COI phylogeny. Radular characters, especially the number of cusps of the outer marginal teeth, are considered valuable in the classification of *Bakyietaia* species with similar shells. The COI phylogeny revealed that three endemic species of Hainan Island form a small non-adaptive radiation and have an origin from Leizhou Peninsula. Two *Bakyietaia* species with a disjunct island – mainland distribution hint towards a major role for marine regression and transgression in the South China Sea since Pleistocene in shaping the current distributions of *Bakyietaia* species.

ADDITIONAL KEYWORDS: biogeography, gastropod, insular radiation, systematics, taxonomy.

INTRODUCTION

Viviparidae (river snails) is a family of freshwater snails comprising 125 to 150 valid described extant species with a worldwide distribution except for South America and Antarctica (Strong *et al*., 2008). There is a rich fossil record of Viviparidae (Prashad, 1928), and many extinct species are found out of the current geographic range of this family, such as in the Middle East (Sivan *et al*., 2006) and South America (Ghilardi *et al*., 2011). The oldest fossils can be traced back to Early to Middle Jurassic: *Viviparus sangongheensis* Zhu, 1994 † from Xinjiang, China (Early Jurassic; Zhu, 1994) and *Bathonella scotica* (Tate, 1873) † from England, UK (Middle Jurassic; Tracey *et al*., 1993). The early origin, abundant fossil record and (almost) worldwide distribution of Viviparidae has drawn many researchers' attention to the systematics, evolutionary history and biogeography of this family (Sengupta *et al*., 2009; Schultheiß *et al*., 2014; Zhang *et al*., 2015; Hirano *et al*., 2019a; Ye *et al*., 2020; Stelbrink *et al*., 2020; Hirano *et al*., 2023).

South China and Southeast Asia harbour the highest biodiversity of Viviparidae (Strong *et al*., 2008; Zhang *et al*., 2015; Stelbrink *et al*., 2020). Yunnan Province of Southwestern China harbours ten genera, including four endemic ones (*Anularya* Zhang & Chen, 2015; *Dalipaludina* Zhang, 2023; *Margarya* Nevill, 1877; *Tchangmargarya* He, 2013) and at least 15 endemic species (Zhang *et al*., 2023). Southeast Asia harbours altogether 12 genera, including four endemic ones from mainland Southeast Asia (*Anulotaia* Brandt, 1968; *Eyriesia* Fischer, 1885; *Taia* Annandale, 1918; *Trochotaia* Brandt, 1974) and two endemic ones from insular Southeast Asia (*Celetaia* Clench, 1966; *Torotaia* F. Haas, 1939) (Molluscabase eds., 2023). However, Viviparidae from this region are clearly understudied, with only a few recent studies focussing on this hotspot of viviparid diversity (Zhang *et al*., 2015; Stelbrink *et al*., 2019; Stelbrink *et al*., 2020; Zhang *et al*., 2023).

Rao (1925) erected a new genus of Viviparidae, *Dactylochlamys*, for a single species with many smooth strong keels on shell endemic to the Manipur Valley of India, *Paludina oxytropis* Benson, 1836. However, Rao (1931) later found out that *Dactylochlamys* Rao, 1925 should be a junior homonym of the ciliate *Dactylochlamys* Lauterborn, 1901; therefore, *Angulyagra* Rao, 1931 was erected as a replacement name for this genus. After the description of the genus, *Angulyagra* soon became a "wastebasket taxon". A lot of viviparid species from Asia were described from or assigned to this genus just because they possess a shell with many smooth strong keels (Prashad, 1928; Yen, 1939; Yen, 1943; Starobogatov, 1970; Liu et al., 1995). However, most of these *Angulyagra* species are obviously distinct from the type species of *Angulyagra* based on characters of the shell, radula, operculum, and anatomy. Therefore, a systematic revision of *Angulyagra* is needed.

Here, we systematically revise the *Angulyagra* species from South China and Mainland Southeast Asia based on their morphology (shell, operculum, radula), ecology, distribution and variation in the mitochondrial COI gene. Our integrative study has revealed that there are at least 18 species within this group, and all of these *Angulyagra* species should actually be assigned to a new genus described here: *Bakyietaia* gen. nov.. The evolutionary history and biogeography of *Bakyietaia* species are discussed based on the molecular phylogeny and their distribution.

MATERIAL AND METHODS

MATERIAL

Type specimens of ten formerly described Viviparidae species from four institutions (Natural History Museum, London (BMNH); Gymnasium der Franziskaner, Bozen (GFBI); Naturhistorisches Museum, Wien (NHMW); Naturmuseum Senckenberg, Frankfurt (SMF); Muséum national d'Histoire naturelle, Paris (MNHN)) have been examined. The type specimens of nine new species described here have been deposited in the following collections: Zujia Biological Museum of Fudan University, Shanghai (ZBMFD), Museum für Naturkunde, Berlin (ZMB) and the collection of Le-Jia Zhang (ZLJ). The non-type specimens kept in ZMB have been examined. The specimens used for the morphological and molecular analysis were collected during 2006-2022. A two-month field work for collecting Viviparidae species was conducted in the rivers, springs, lakes and wetlands of Southern China in early 2022. Altogether 73 sampling sites where *Bakyietaia* species were collected are illustrated in the map (Fig. 1). The newly collected specimens are kept in ZMB and ZLJ.

EXAMINATION OF MORPHOLOGY

The height (H) and width (W) of shells of mature and complete specimens were measured with an electronic calliper to a precision of 0.1 mm. The mass (M) of several selected shell specimens was measured by an electronic scale to a precision of 0.01 g. The shells were

completely dry and the mass was constant, including nine specimens of *Angulyagra oxytropis* and 44 specimens of *Bakyietaia* species. All specimens were photographed in a consistent orientation using a Sony alpha 7R 4 camera. Radulae were extracted through dissection, and cleaned by boiling in 1% NaOH solution for half an hour and rinsed with distilled water. Radulae were coated with gold before taken photos with a Zeiss EVO LS10 scanning electron microscope. The terms used for describing the operculum were according to Zhang & von Rintelen (2021).

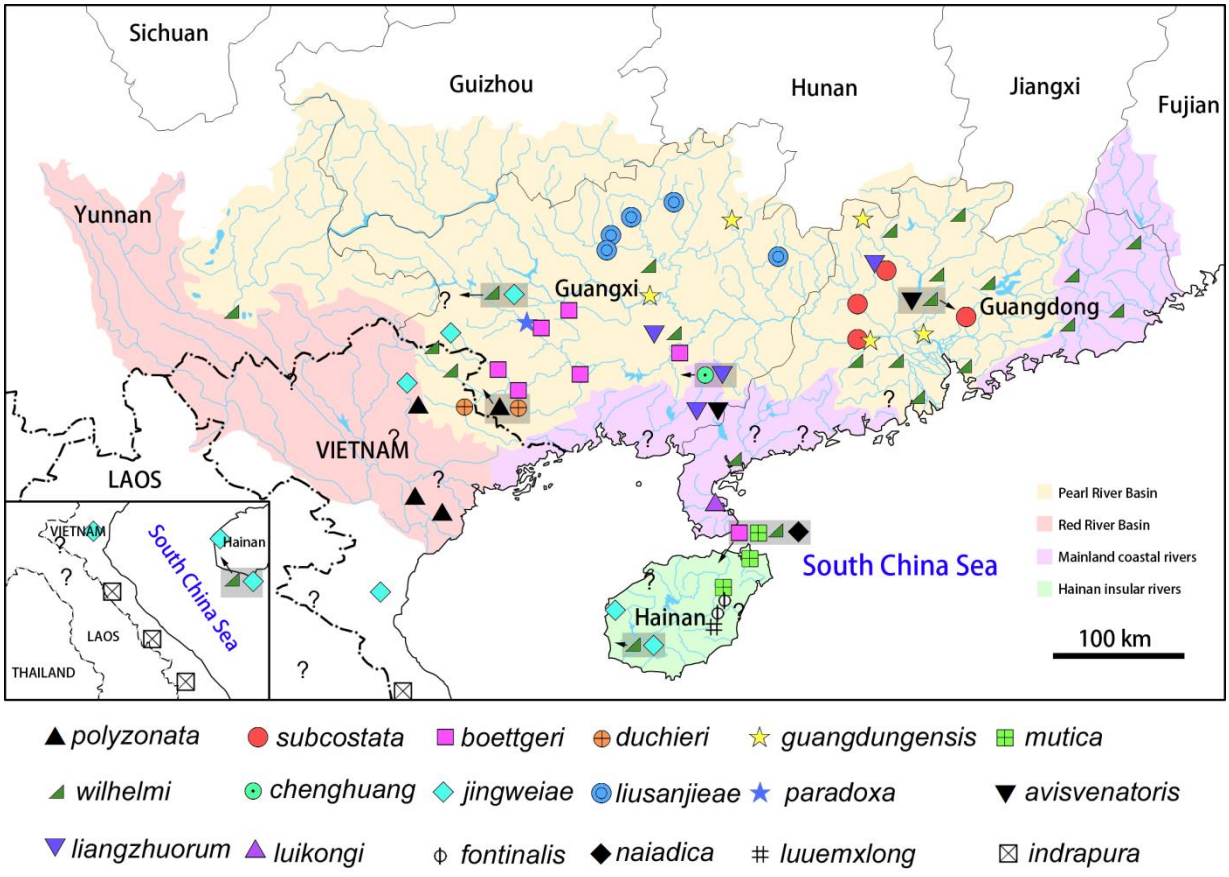


Figure 1. Map of the main river basins in South China and mainland Southeast Asia with the collecting sites of *Bakyietaia* species. The sites with more than one species are pointed by an arrow and several symbols with grey background. The symbols "?" refer to the sites with unidentified *Bakyietaia* species.

MOLECULAR METHODS

DNA was extracted from 10-20 mg foot tissue of each snail, using a mollusc-specific CTAB/chloroform extraction protocol (Winnepenninckx *et al.*1993). A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified through polymerase chain reaction (PCR) with the following primer pair: LCO1490, 5′-

GGTCAACAAATCATAAAGATATTGG-3′ and COX-B7R, 5′-ACCACCAGCTGGATCAAAAA-3′. (Schultheiß *et al*. 2011) PCR amplifications were conducted in 25 µl volumes under the following cycling conditions: initial denaturing step at 94 °C for 10 min, followed by 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min, with a final extension step of 10 min at 72 °C. The purification and sequencing were conducted by Macrogen Europe, Amsterdam, Netherlands.

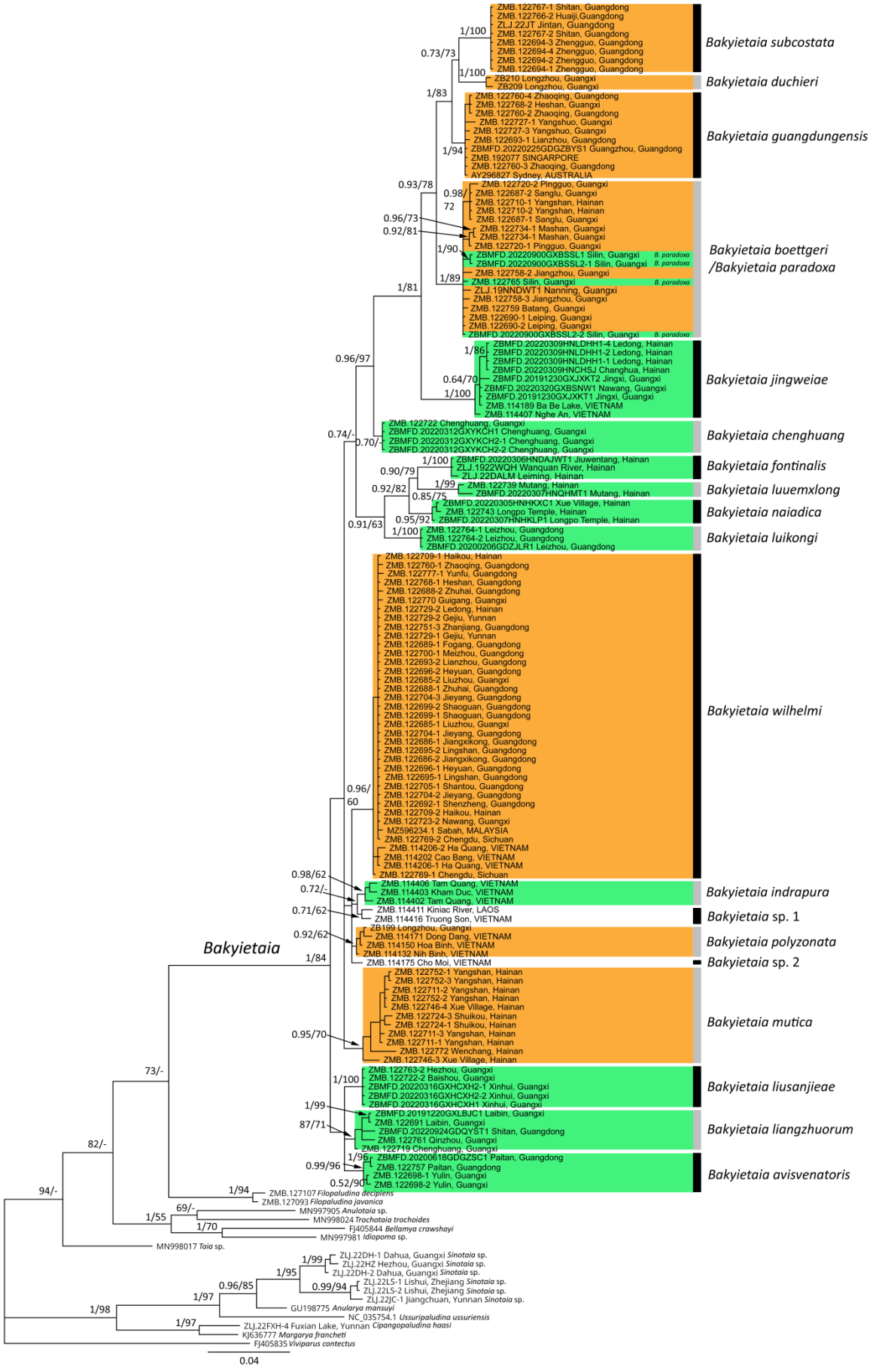

Bakyietaia subcostata
Bakyietaia duchieri
Bakyietaia guangdungensis
Bakyietaia boettgeri /Bakyietaia paradoxa
Bakyietaia jingweiae
Bakyietaia chenghuang
Bakyietaia fontinalis
Bakyietaia luuemxlong
Bakyietaia naiadica
Bakyietaia luikongi
Bakyietaia wilhelmi
Bakyietaia indrapura
Bakyietaia sp. 1
Bakyietaia polyzonata
Bakyietaia sp. 2
Bakyietaia mutica
Bakyietaia liusanjieae
Bakyietaia liangzhuorum
Bakyietaia avisvenatoris
Bakyietaia
0.04

Figure 2. Bayesian inference phylogram based on cytochrome c oxidase subunit I (COI) sequences of the selected species of Viviparidae, with an emphasis on the *Bakyietaia* species. Numbers above branches are BI posterior probabilities/ML bootstrap values. The clades with orange background are new combinations and the clades with green background are new species.

SEQUENCE AND PHYLOGENETIC ANALYSES

140 new COI sequences of Viviparidae species have been uploaded to Genbank (Table S1). In addition, 16 sequences of 13 genera of the subfamily Bellamyinae were included in the present study, mostly from Stelbrink *et al*. (2020). *Viviparus contectus* of subfamily Viviparinae was selected as outgroup based on Stelbrink *et al*. (2020).

Sequences were aligned using MUSCLE as implemented in Geneious Prime 2020 (https://www.geneious.com). Genetic distances were calculated using MEGA X (Kumar *et al*. 2018). TN93+G+I was suggested as the best-fit model of sequence evolution for our dataset by means of the Akaike and Bayesian information criteria in MEGA X. A Bayesian inference (BI) analysis was performed with MrBayes 3.2.6 (Ronquist *et al*. 2012) as implemented in Geneious Prime 2020 with four independent chains for 5,000,000 generations, sample freq = 1,000, burnin = 25%, and it was confirmed that convergence was reached based on the trace plots generated in Geneious Prime 2020. A maximum likelihood (ML) analysis was conducted with RAxML as implemented in Geneious Prime 2020, with support estimated by 1,000 bootstrap replicates (Alexey *et al*., 2019).

RESULTS

GENETIC DIFFERENTIATION AND PHYLOGENY

The COI p-distances within one species of *Bakyietaia* are 0-1.42%; the COI p-distances between different species of *Bakyietaia* are 1.68%-7.53% (except for one between *B. boettgeri* and *B. paradoxa*, 0-1.08%); the COI p-distances between *Bakyietaia* and other genera of Viviparidae are 8.45%-15.65%.

The phylogenetic trees reconstructed by BI and ML based on COI are highly congruent, therefore only the BI tree is displayed (Fig. 2). The new genus *Bakyietaia* is monophyletic and distinct from all other included genera of Viviparidae. Most the 16 identified species of *Bakyietaia* are recovered as monophyletic with high support values, except for *Bakyietaia boettgeri* and *Bakyietaia paradoxa*, which are clustering together within a comb-like clade. Notably, the three endemic species from the Hainan Island (*B. fontinalis*, *B. naiadica*, *B. luuemxlong*) cluster together in a monophyletic clade, and *B. luikongi* endemic to the Leizhou Peninsula is sister to this Hainan clade. *Bakyietaia* is part of a larger clade including the genera *Filopaludina*, *Bellamya*, *Idiopoma*, *Anulotaia*, *Trochotaia* and *Taia*.

STATISTICS OF SHELL CHARACTERS

The shell measurements reveal that *Angulyagra oxytropis* has a larger, more compacted but lighter shell compared to *Bakyietaia* species. The height of *Angulyagra oxytropis* is larger than that of any *Bakyietaia* species, and the larger W/H of *Angulyagra oxytropis* shows that it has a relatively more compacted shell regardless of size (Table 1, Fig. 3A). *Angulyagra oxytropis* always has a larger shell than *Bakyietaia* species when the mass of both is the same; correspondingly, *Angulyagra oxytropis* always has much lighter shell than *Bakyietaia* species when the height of both is the same (Fig. 3B).

Tabel 1. Shell measurements of *Angulyagra* and *Bakyietaia* species.

Species	*N*	*n*	H (mm)	W(mm)	W/H	M (g)
Angulyagra oxytropis	9	9	34.60±3.77	27.15±3.79	0.78±0.03	1.01±0.28
Bakyietaia polyzonata	3		26.60±1.91	19.19±1.30	0.72±0.01	-
Bakyietaia subcostata	10		27.17±3.64	20.25±1.75	0.75±0.05	2.52±0.36
Bakyietaia boettgeri	9	7	26.76±6.67	18.87±5.12	0.70±0.04	2.16±2.78
Bakyietaia duchieri	2		32.88±1.00	24.46±1.77	0.74±0.03	-
Bakyietaia guangdungensis	7	3	20.87±0.93	14.57±0.61	0.70±0.03	0.78±0.18
Bakyietaia mutica	11	2	22.14±1.74	15.49±1.06	0.70±0.04	0.84±0.16
Bakyietaia wilhelmi	10	10	25.53±3.72	17.28±2.11	0.68±0.04	1.63±0.97
Bakyietaia chenghuang	3	2	23.70±5.37	17.67±3.42	0.74±0.03	1.19±0.02
Bakyietaia jingweiae	9	9	26.79±5.37	17.67±3.42	0.66±0.04	1.94±1.90
Bakyietaia liusanjieae	12	1	25.69±3.35	18.98±2.31	0.74±0.04	3.00
Bakyietaia paradoxa	7		25.46±1.19	18.72±0.92	0.74±0.02	-
Bakyietaia avisvenatoris	6		19.78±1.67	14.50±1.48	0.73±0.03	-
Bakyietaia liangzhuorum	8	4	25.25±3.07	18.13±2.33	0.72±0.04	0.77±0.22
Bakyietaia luikongi	3		20.66±0.70	15.44±0.62	0.75±0.02	-
Bakyietaia fontinalis	8	1	24.25±2.59	17.44±1.84	0.72±0.03	0.40
Bakyietaia naiadica	3		25.73±0.62	18.02±0.31	0.70±0.01	-
Bakyietaia luuemxlong	4		23.56±2.58	16.49±1.29	0.70±0.02	-
Bakyietaia indrapura	3	3	26.92±2.45	17.67±1.40	0.66±0.01	1.07±0.41

Values are arithmetic means and standard deviations of height (H) and width (W) of *N* measured specimens and mass (M) of *n* measured completely dry shells.

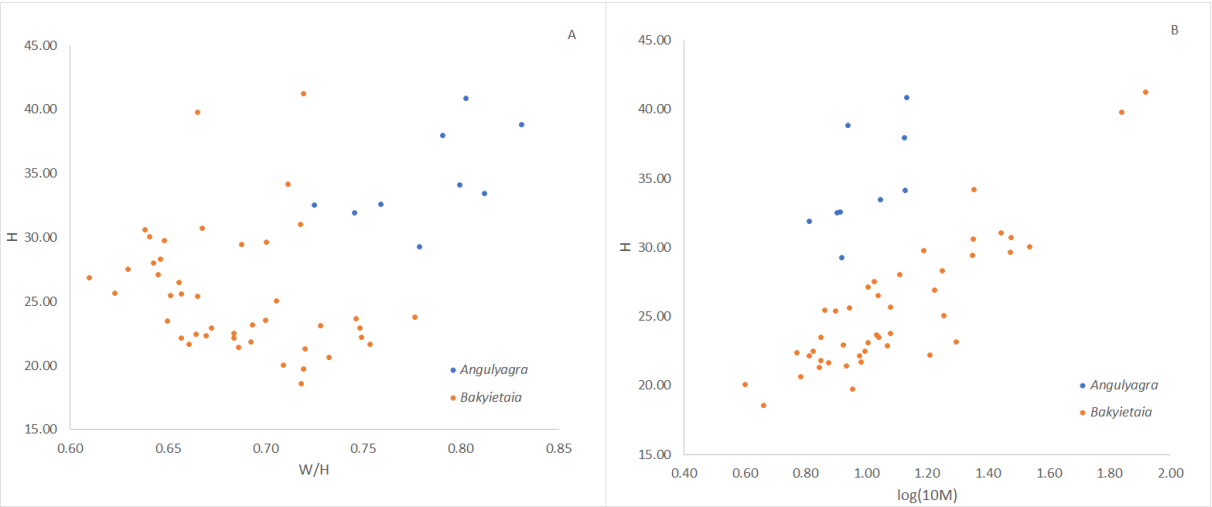


Figure 3. Comparison of *Bakyietaia* species with respect to parameters H and W/H (A), and with respect to parameters H and log (10M) (B).

SYSTEMATICS

FAMILY VIVIPARIDAE J. E. GARY, 1847

SUBFAMILY BELLAMYINAE ROHRBACH, 1937

ANGULYAGRA RAO, 1931

Dactylochlamys Rao, 1925: 132 [non Lauterborn, 1901, junior homonym].

Angulyagra Rao, 1931: 301

Type species: *Paludina oxytropis* Benson, 1836 (original designation)

Original diagnosis

"The shell is of large size, conical, thin but ornamented with prominent smooth spiral ridges which are concave on the internal surface. The base is somewhat flattened. The umbilicus is broadly rimate with abroad channel descending downwards from it. The mantle has enlarged and highly vascular finger-shaped processes on the margin" (Rao, 1925).

Remarks

The shell of *Angulyagra* is most similar to that of *Torotaia* Haas, 1939 or *Dalipaludina* Zhang, 2023, since all of them have a large thin shell with strong keels, acute apex, and simple inner lip. It can be distinguished from these two genera based on the following combination of characters: teleoconch whorls with strong keels obviously darker than the other part of shell surface in colour, open umbilicus bordered by a strong keel; operculum with growth lamellae on exterior surface and radial striae on interior surface; radula with narrow marginal teeth, both inner and outer marginal teeth only half of lateral tooth in width, and outer marginal teeth with 15 tiny cusps; highly vascular finger-shaped processes on the mantle margin.

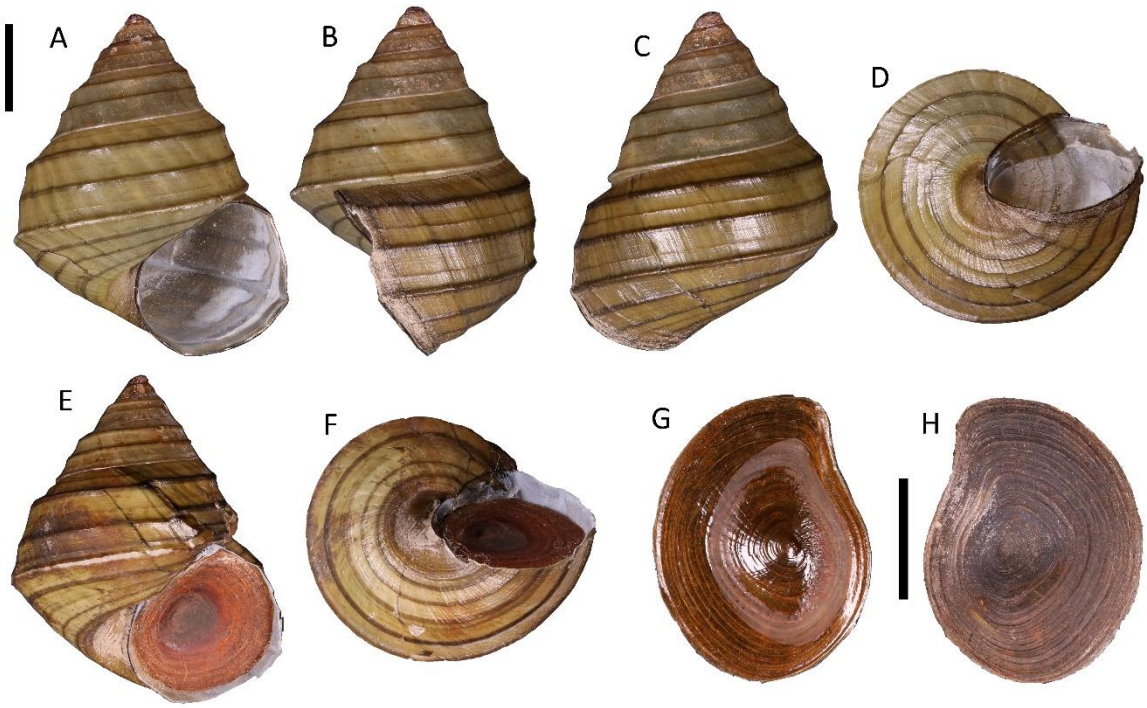


Figure 4. Shells and operculum of *Angulyagra oxytropis*: A-D, BMNH.2019.04.31.1, Loktak Lake, India; E-F, BMNH.2019.04.31.2, Loktak Lake, India; G-H, operculum of BMNH.2019.04.31.1; scale bar=1 cm, All shells & operculum G-H separately with the same scale bar.

ANGULYAGRA OXYTROPIS (BENSON, 1836)

Paludina oxytropis Benson, 1836: 745 (Type locality not stated in the original description).

Paludina pyramidata von dem Busch, 1844: 113, pl. 1, figs. 3, 4.

Vivipara oxytropis – Preston, 1915: 84; Annandale, 1920: 113, Annandale, 1921: 544–552, figs. 2–5.

Dactylochlamys oxytropis – Rao, 1925: 132.

Angulyagra oxytropis – Rao, 1931: 301; Subba Rao, 1989: 51, fig. 70; Preece *et al*., 2022: 94, fig. 38A.

Material examined

Holotype: *Paludina oxytropis*, BMNH.1879.12.26.120, with a label “Bengal”. Paratypes: *Paludina* oxytropis, BMNH.1974.03.7.1- BMNH.1974.03.8, 8 dry shells (6 adults and 2 juveniles), “Logtak Lake, Manipur”.

Additional material: *Paludina oxytropis*, BMNH.2019.04.31, 2 dry shells, “Loktak Lake, Manipur, Assam”.

Description

Shell (Fig.4A-F) large, broadly conical, thin, light weight, fragile, olive to greenish brown; up to six whorls at adulthood, including one relatively smooth protoconch whorls, apex acute; each teleoconch whorls with three strong spiral keels in darker colour, the keel above suture strongest, one to two weaker spiral threads located between strong keels; base of shell with three to four spiral keels in darker colour, one to two weaker spiral threads located between strong keels; aperture ovate, inner shell surface bluish white, with obvious grooves where the three strong keels located, outer lip thin, three strong keels extending to the margin of outer lip, inner lip thin and simple; umbilicus broadly open, bordered by a strong keel.

Operculum (Fig.4G-H) corneous, transparent pale brown, rather thin; nucleus sub-central, close to inner margin; exterior surface with regularly distributed growth lamellae; interior surface with relatively large inner opercular region and radial striae starting from nucleus; nuclear region relatively large, smooth and glossy, with veins at margin.

Radular central tooth with one broad central denticle and five small sharp cusps on either side; lateral tooth with one broad central denticle, three small sharp cusps on inner side and six small sharp cusps on outer side; inner and outer marginal teeth narrow, both nearly half of lateral tooth in width; inner marginal tooth with one broad central denticle and three small sharp cusps on either side; outer marginal tooth with 15 tiny sharp cusps (summarized based on the figure and description from Annandale, 1921).

Mantle edge with a number of finger-shaped processes, three of these processes larger, each large process corresponding to the grooves of inner shell surface; gill lamella with a broad base and a long finger-shaped part; about ten embryos and ten eggs in the uterus of pregnant female (summarized based on the figure and description from Rao, 1925 and Annandale, 1921).

Remarks

This species can be easily distinguished from the other viviparid species according to the remarks for the genus. As the type species of *Angulyagra*, *A. oxytropis* shows large morphological and anatomical differences to many species nominally assigned to *Angulyagra*, especially in the large, thin, light and fragile shell with an open umbilicus and simple inner lip, the distinct operculum, and marginal teeth of the radula.

Habitat and distribution

Loktak Lake and nearby wetland ponds in the Manipur Valley of India.

BAKYIETAIA ZHANG, YEN & VON RINTELEN **GEN. NOV.**

Type species: *Vivipara polyzonata* Frauenfeld, 1862

Etymology: "Bakyie" is derived from "Bakyied" in Zhuang Language ("百越" in Chinese), referring to various ethnic groups living in South China and Northern Vietnam; "taia" refers its similarity to the viviparid genus *Taia* Annandale, 1918. The recommended Chinese name is "越田螺".

Diagnosis

Shell mostly medium in size, thin to thick, always solid, conical; apex acute, teleoconch whorls with three smooth main keels, with one to two weaker smooth keel located between suture and the first keel, base of shell with one to six obvious keels, keels usually darker than the other part of shell surface in colour; inner lips from different growing periods outward folding and tightly overlapping, sometimes forming a crescent callus-like structure, completely covering umbilicus; operculum with smooth exterior surface and a relatively small nuclear region with shiny grains or veins; radula with a narrow inner marginal teeth (smaller than lateral tooth in width) and a wide outer marginal teeth (almost same as lateral tooth in width), outer marginal teeth with 15 to 30 tiny cusps; mantle margin simple and smooth.

Remarks

Previously described species of this genus have hitherto mostly been assigned to the genus *Angulyagra*, while a few have been assigned to the genera *Taia* or *Sinotaia*. *Bakyietaia* species can be easily distinguished from *Angulyagra* species based on their smaller, more solid and heavier shell with a crescent callus-like structure of the inner lip covering the umbilicus; the characters of the operculum and the radula of *Bakyietaia* are obviously distinct from those of *Angulyagra*; we did not observe the "finger-shaped processes on the margin" of the mantle of *Angulyagra* in the smooth mantle margin of *Bakyietaia* species. *Sinotaia, Anulotaia* and some species of *Filopaludina* have a similar medium-sized and solid conical shell (Fig. 5). *Bakyietaia* can be distinguished from these genera based on the following combination of characters: teleoconch whorls with three smooth main keels and one to two weaker keels, mostly darker than the other part of shell surface in colour; inner lips from different growing periods outward folding and tightly overlapping, usually completely covering umbilicus; inner marginal teeth narrow, outer marginal teeth wide and with 15 to 30 tiny cusps. The COI tree supports that *Bakyietaia* is a distinct lineage from *Taia*, *Sinotaia*, *Anulotaia* and *Filopaludina*.

Habitat and distribution

Found in many kinds of lentic and lotic freshwater habitats (Fig. 6). Native to southern China, northern to central Vietnam and Laos.

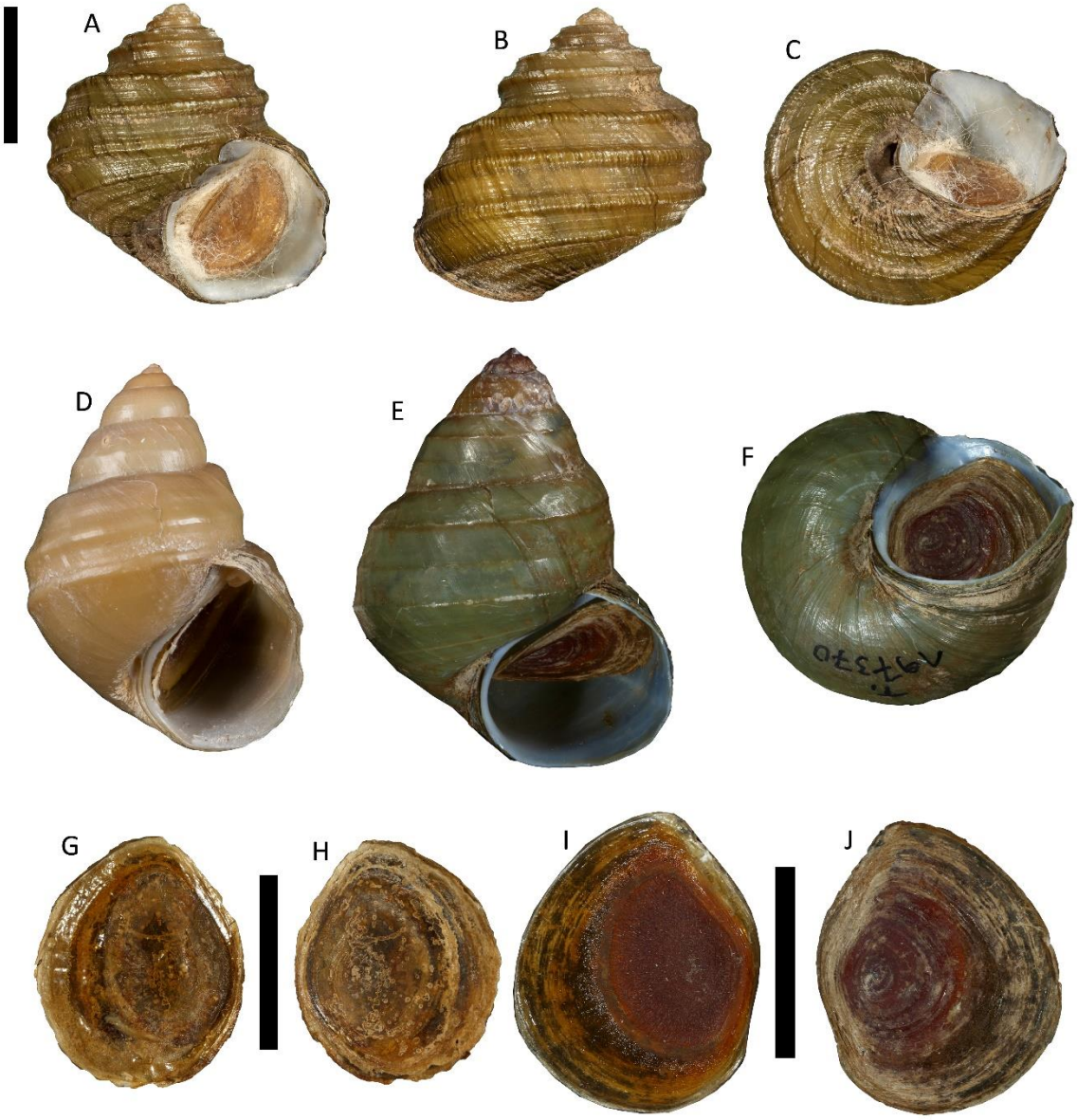


Figure 5. Shells and operculum of Viviparidae species for comparison: A-C, *Anulotaia forcarti* Brandt, 1968, SMF.197369, Maenam Mun, Thailand; D, *Sinotaia* sp., ZLJ.2208DH, Dahua, Guangxi, China; E-F, *Filopaludina maekoki* Brandt, 1968, SMF.197370, Ban Mae Chai, Thailand; G-H, operculum of *Anulotaia forcarti*, SMF.193416; I-J, operculum of *Filopaludina maekoki*, SMF.197370; scale bar of all shells and operculum I-J =1 cm, scale bar of operculum G-H = 0.5 cm.



Figure 6. Habitat (A-F) and living animals (G-I) of *Bakyietaia* species. A. Xi River near Xiaoxiang Town, Guangdong, China; B, spring pond near Longpo Temple, Hainan, China; C, Yangshan Reservoir, Hainan, China; D, Yangshan Wetland, Hainan, China; E, Baishou River, Guangxi, China; F, concrete canal in Zhadian Village, Yunnan, China; G, *Bakyietaia guangdungensis* from Yangshuo, Guangxi, China; H, *Bakyietaia chenghuang* from Chenghuang Town, Guangxi, China; I, *Bakyietaia liangzhuorum* from Chenghuang Town, Guangxi, China.

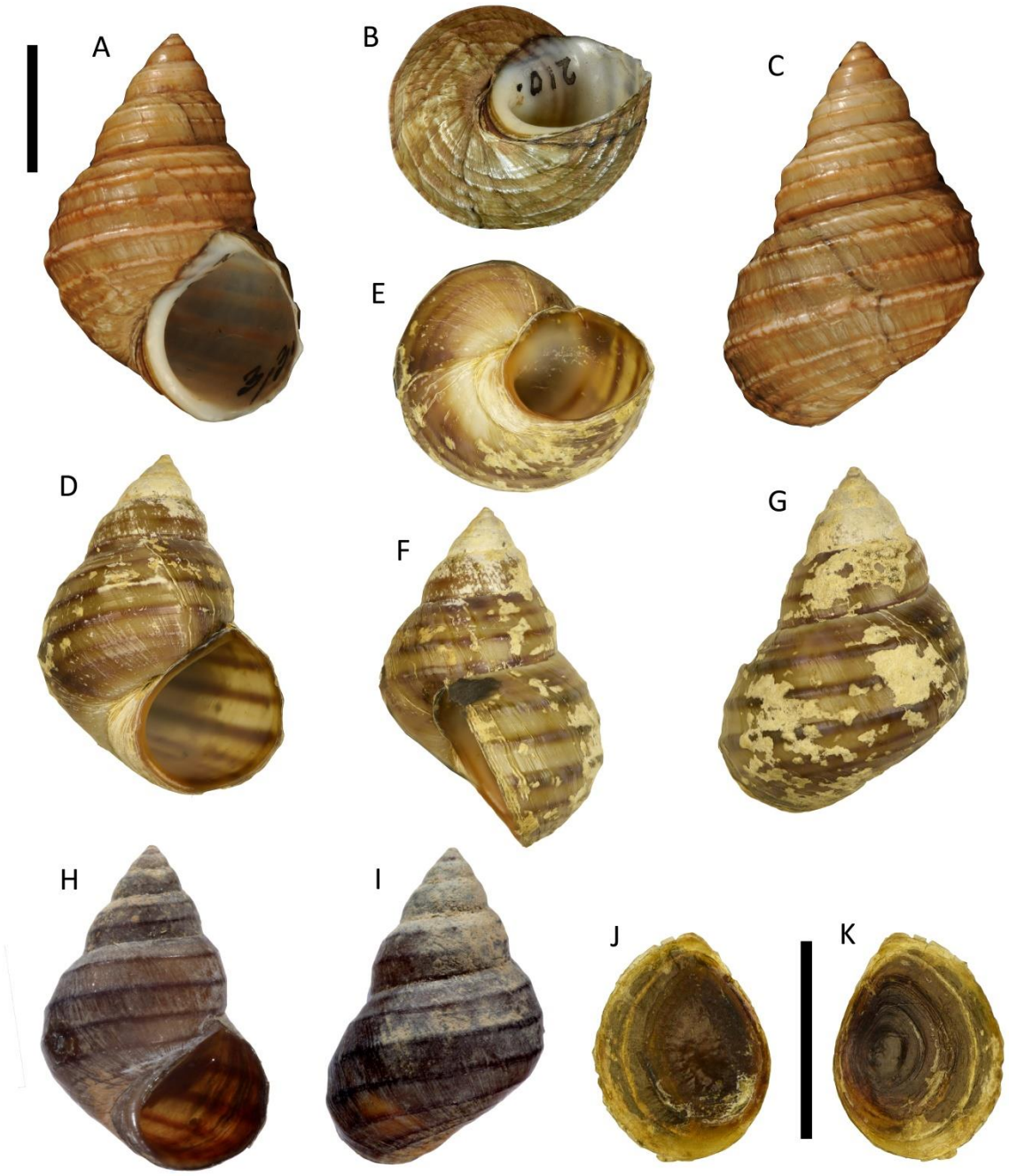


Figure 10. Shells and operculum of *Bakyietaia polyzonata*: A-C, lectotype, NHMW 68037, photos by A. Schumacher and I. Gallmetzer; D-G, ZB199, Longzhou, Guangxi, China; H-I, ZMB.114171, Deng Dang, Vietnam; J-K, operculum of ZB199; scale bar=1 cm, All shells & operculum J-K separately with the same scale bar.

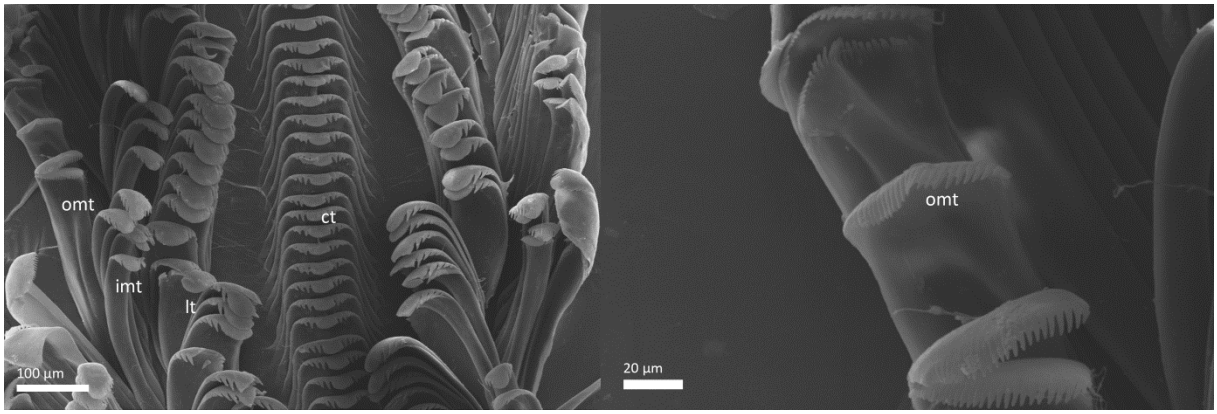


Figure 12. SEM photo of the radula of *Bakyietaia polyzonata*, ZMB.114171. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA POLYZONATA (FRAUENFELD, 1862) **COMB. NOV.**

Vivipara polyzonata Frauenfeld, 1862: 1165-1166 (Type locality not stated in the original description).

Paludina polyzonata – Fischer & Dautzenberg, 1904: 92; Dautzenberg & Fischer, 1908: 199.

Angulyagra polyzonata – Thanh *et al*., 2004: 3–4, fig. 4; Richter 2015: 127–128, fig. 3.5.

Angulyagra boettgeri – Madsen & Hung 2014: 109, figs. 2 (10 right) [non Fischer, 1898].

Material examined

Lectotype: *Vivipara polyzonata*, NHMW68037, designated by Yen (1939). Paralectotypes: *Vivipara polyzonata*, NHMW68038, 1 specimens.

Additional material (Fig.11): ZMB.114132, 10 living-collected specimens, Cuc Phuong N.P., Reservoir nr Dang, Ninh Binh, Vietnam; ZMB.114150, 1 living-collected specimens, Ngoc Ly, Tan Lac district , Hoa Binh, Vietnam; ZMB.114171, 3 living-collected specimens, Dong Dang, Yen Ninh, Phu Luong, Bac Kan, Vietnam; ZB198-ZB200, 3 living-collected specimens, outlet of an underground river in a cave, near Poliu Village, Shuikou Town, Longzhou County, Chongzuo City, Guangxi Province, China, 4.3.2023.

Description

Shell (Fig.10A-J) medium, thin but solid, conical, olive to greenish brown, with five to six whorls at adulthood, apex acute; teleoconch whorls, especially body whorl, slightly inflated, sometimes forming shoulder, with three smooth main keels, with one to two weaker smooth keel located between suture and the first keel, sometimes one additional weak keel located between first and second/second and third keels, base of shell with three to five weak keels, keels usually darker than the other part of shell surface in colour; aperture ovate, less than half

of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thick nacreous layers, white or orange, inner lips of the last growing stages outward folding and tightly overlapping, forming a very narrow crescent callus-like structure or without this structure, inner lip or this structure usually covering umbilicus, umbilicus sometimes narrowly open, bordered by a keel.

Operculum (Fig.10K-L) corneous, ovate, thin, slightly smaller than shell aperture, yellow to orange; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins.

Radular (Fig.12) (n=4) central tooth with one broad central denticle and five small sharp cusps on either side; lateral tooth with one broad central denticle and five small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and five to six small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 21 to 26 small sharp cusps.

Remarks

The newly collected specimens from Nghe An of Vietnam have a shell that is highly similar to that of the lectotype. *Bakyietaia polyzonata* can be distinguished from most congeners by its medium-sized thin conical shell with inflated whorls. The type locality of this species was not clear from the original description, and it was considered as China (Yen, 1939). However, this species has never been found in China based on historic and recent materials. The distribution of *Bakyietaia polyzonata* in central Vietnam and Laos is far from the distributions of other congeners. It is probably the southernmost native species of this genus. The recommended Chinese name is “多棱越田螺”.

Habitat and distribution

Rivers and lakes of Western Guangxi Province of China and Northern Vietnam.

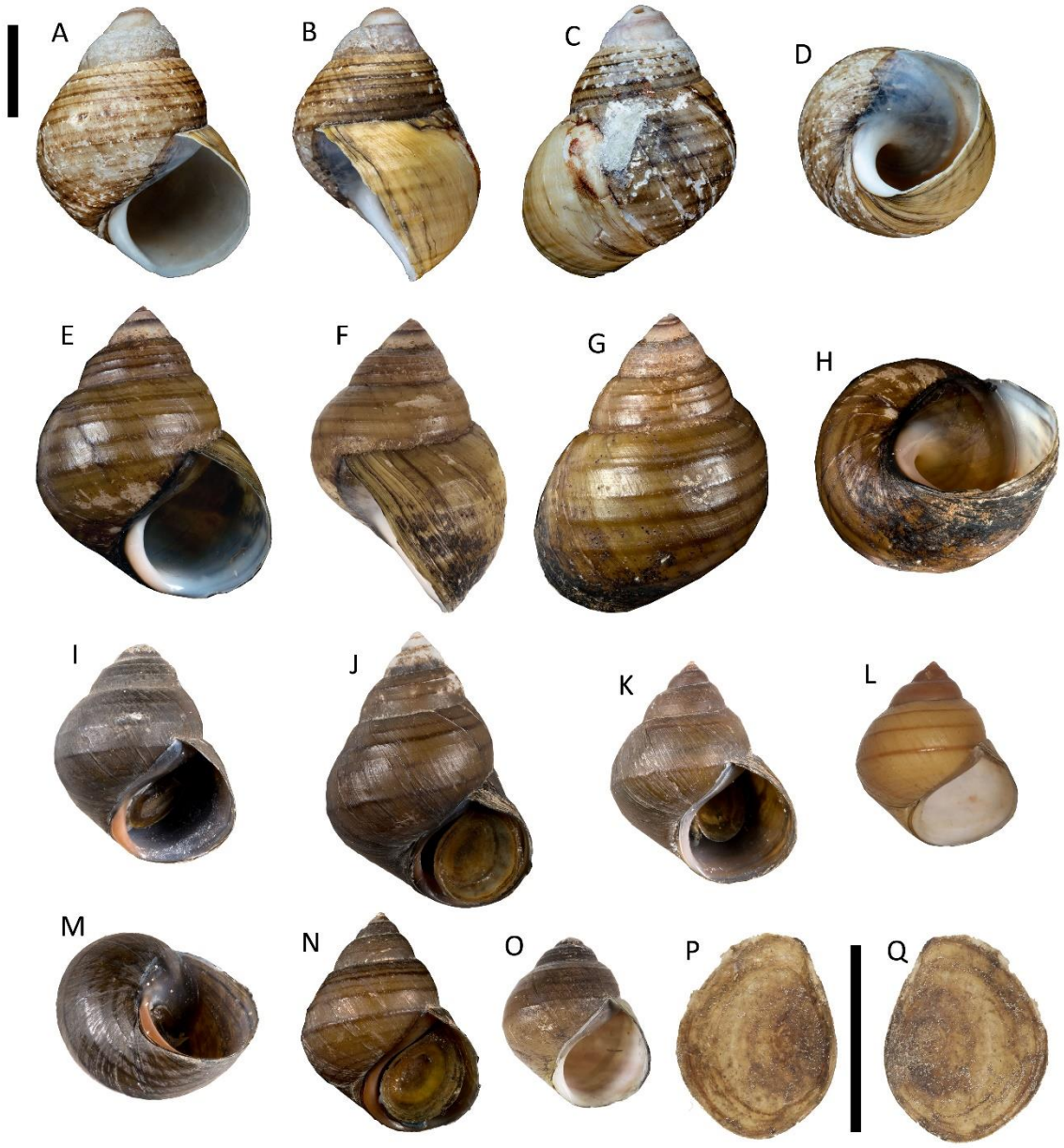


Figure 7. Shells and operculum of *Bakyietaia subcostata*: A-D, holotype, BMNH.2007.01.7.1, China, photo by Jonathan Ablett; E-H, ZLJ.21XX, Xiaoxiang Town, Guangdong, China; I & M, ZMB.122766, Huaiji County, Guangdong, China; J & N, ZMB.122767, Shitan Town, Guangdong, China; K, ZLJ.22JT, Jintan Town, Guangdong, China; O, ZMB.122694, Zhengguo, Guangdong, China; L, holotype of *Paludomus futaii*, GFBI.22, Bei River, Guangdong, China; P-Q, operculum of ZLJ.21XX; scale bar=1 cm, All shells & operculum P-Q separately with the same scale bar.

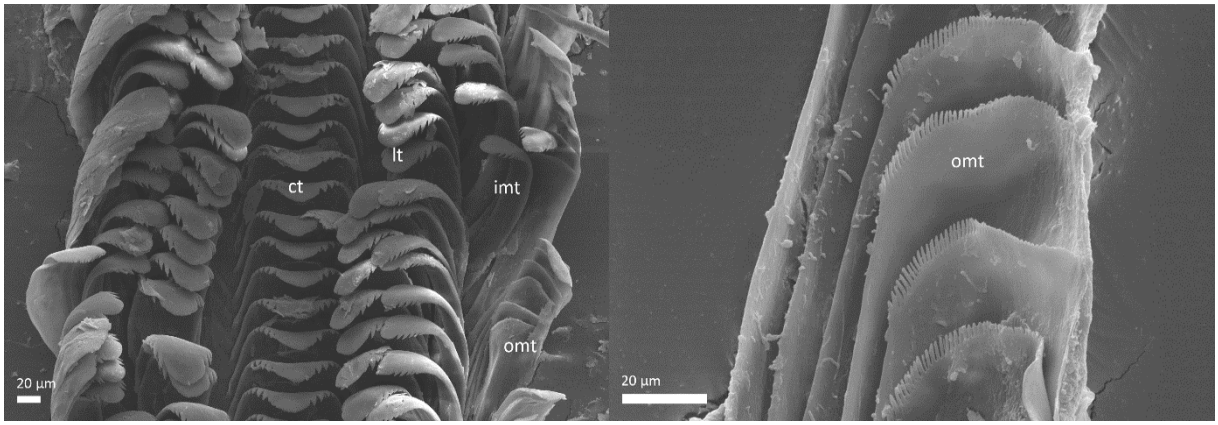


Figure 9. SEM photo of the radula of *Bakyietaia subcostata*, ZMB.122766. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA SUBCOSTATA (GRAY, 1833) **COMB. NOV.**

Paludina subcostata Gray, 1833 in Griffith & Pidgeon, 1833-1834: 599, pl. 36, fig. 3 ("China").

Paludomus futaii Gredler, 1889: 159 ("Peho, Kuang-tung", refers to Bei River, Guangdong Province, China).

Sinotaia? *futaii* – Zilch, 1974: 64, pl. 7, fig.9.

Angulyagra subcostata – Liu *et al*., 1995: 14.

Material examined

Holotype: *Paludina subcostata*, BMNH.2007.01.7.1

Holotype of *Paludomus futaii*, GFBI.22.

Additional material (Fig.8): ZMB.122694, 4 living-collected specimens, Zeng River, Zhengguo Town, Guangzhou City, Guangdong Province, China, 0.7 m deep under rock, 25.9.2019, leg. Da-Ming Shi; ZLJ.21XX 3 dead shells, under rock, Xi River, Xiaoxiang Town, Zhaoqing City, Guangdong Province, China, 8.2021, leg. Ji-Lin Jiang; ZMB.122767, 2 living-collected specimens, Bin River, Shitan Town, Qingyuan City, Guangdong Province, China, 0.6 m deep on rock, 24.9.2022, leg. Mr. Chen; ZMB.122766, 2 living-collected specimens, Sui River, Huaiji County, Zhaoqing City, Guangdong Province, China, 15.9.2022; ZLJ.22JT, 1 living-collected specimen, Bin River, Jintan Town, Qingyuan City, Guangdong Province, China, 10.10.2022.

Description

Shell (Fig.7A-O) medium to large, thin but solid, broadly conical or subglobose, olive to dark brown; with five to six whorls at adulthood, apex acute; teleoconch whorls inflated, sometimes forming shoulder, with three smooth weak main keels, with one to two weaker

smooth keel located between suture and the first keel, sometimes one additional weak keel located between first and second/second and third keels, base of shell with three to five weak keels; aperture ovate, large, around half of shell in height, inner shell surface bluish white to purple, outer lip thin and simple, inner lip covered with thick nacreous layers, always whitish orange or pink, inner lips of the last growing stages outward folding and tightly overlapping, forming a very narrow crescent callus-like structure or without this structure, inner lip or this structure completely covering umbilicus.

Operculum (Fig.7P-Q) corneous, ovate, thin, much smaller than shell aperture, yellow; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins.

Radular (Fig.9) (n=4) central tooth with one broad central denticle and five to six small sharp cusps on either side; lateral tooth with one broad central denticle and five small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and four small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 29 to 43 small sharp cusps.

Remarks

Bakyietaia subcostata can be easily distinguished from most congeners based on the subglobose thin shell with weak main keels, a wide aperture and a small operculum. The comparison with morphologically similar species *Bakyietaia chenghuang* sp. can be seen in the remarks of that species. *Paludomus futaii* was described based on a juvenile shell of this species, and it is considered as a junior synonym here. The recommended Chinses name is “次肋越田螺”.

Habitat and distribution

Rock substrate in rivers of Central Guangdong Province, China.

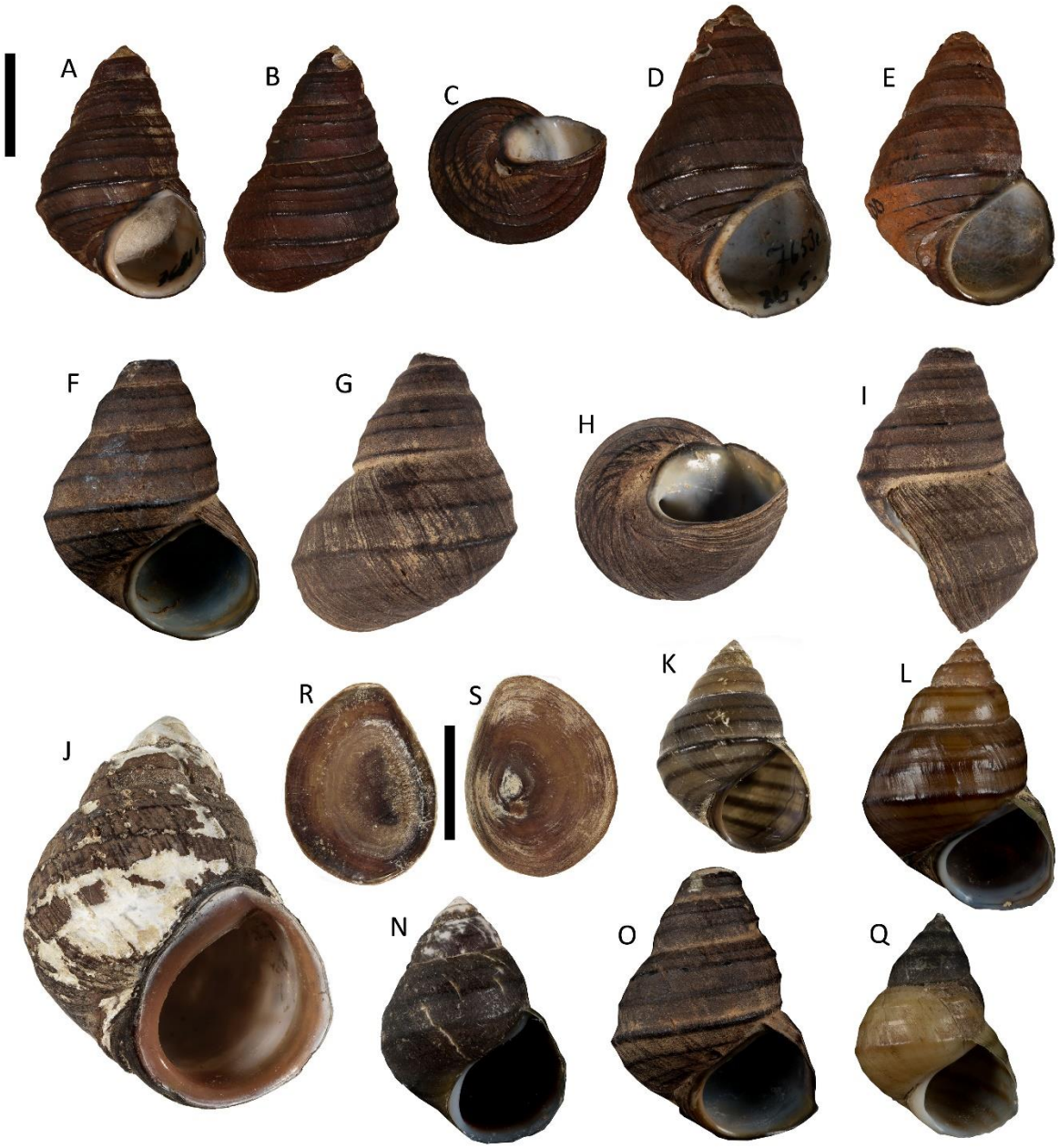


Figure 13. Shells and operculum of *Bakyietaia boettgeri*: A-C, paratype, SMF.7651, Hainan, China; D, paratype, SMF7653, Hainan, China; E, SMF.88400, Hainan, China; F-I, ZMB.122710, Haikou, Hainan, China; J, ZLJ.19NNDWT1, Nanning, Guangxi, China; K, ZMB. 122734, Mashan County, Guangxi, China; L, ZMB.122720, Pingguo, Guangxi, China; N, ZMB.122758, Jiangzhou, Guangxi, China; O, ZMB.122687, Sanglu, Guangxi, China; Q, ZMB.122690, Leiping, Guangxi, China; R-S, operculum of ZLJ.19NNDWT1; scale bars=1 cm, top left bar applies to all shells, separate bar for operculum R-S.

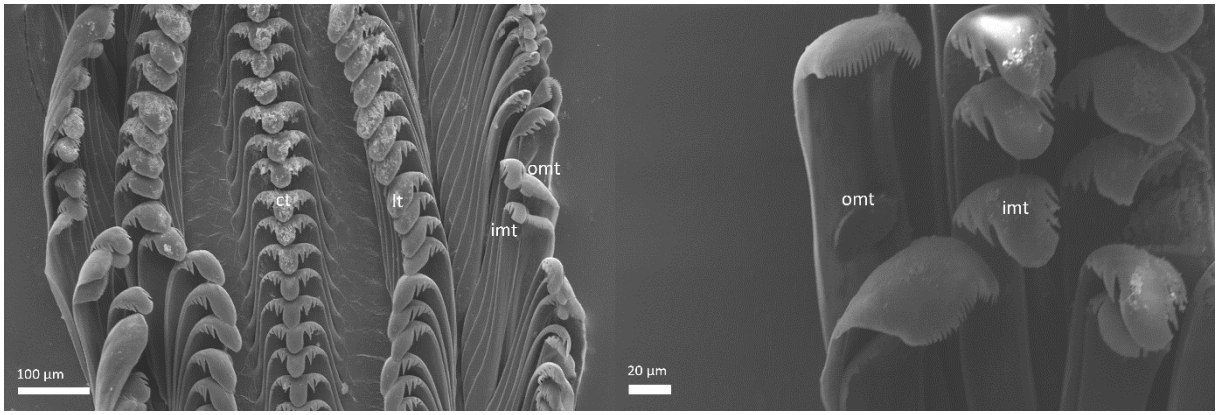


Figure 15. SEM photo of the radula of *Bakyietaia boettgeri*, ZMB.122710. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA BOETTGERI (HEUDE, 1890) **COMB. NOV.**

Paludina boettgeri Heude, 1890: 177, pl. 10, fig. 6 ("Hai-nan", Hainan Province, China).

Vivipara boettgeri – Kobelt, 1909: 137–138, pl. 26, figs. 1–7.

Viviparus (*Taia*) *boettgeri* – Zilch, 1938: 131.

Angulyagra boettgeri – Yen, 1939: 38, pl. 3, fig. 17; Liu *et al*., 1995: 14.

Material examined

Paratypes: *Taia boettgeri*, SMF.7651, 5 dry shells, "S-China: Ins. Hainan", Hainan Island, China, Slg. O. Böttger; *Taia boettgeri*, SMF.7653, 2 dry shells, "S-China: Ins. Hainan", Slg. O. v. Möllendorff.

Additional material (Fig.14): SMF.88400, 2 dry shells, "S-China: Insel Hainan", Slg. Jetschin. ZMB.122710, 5 living-collected specimens, collected from wetlands near Yangshan Reservoir, Haikou, Hainan Province, China, bought by Sheng-Zhuo Huang from the local market on 20.10.2019; ZLJ.19NNDWT1, Dawangtan Reservoir, Nanning City, Guangxi Province, China, 8.2019; ZMB.122687, 5 living-collected specimens, 0.5 m deep on rocks of creeks, Sanglu, Wula Village, Youjiang District, Baise City, Guangxi Province, China, 17.10.2019, leg. Jian-Zhuo Li; ZMB.122759, 5 living-collected specimens, 0.7 m deep on rocks, in a pond near Yapengling, near Batang Village, Gangnan District, Guigang City, Guangxi Province, China, 31.10.2020, leg. Xia-Ming Li; ZMB.122690, 4 living-collected specimens, 1 m deep on rocks, in Heishui River, Leiping Town, Daxin County, Chongzuo City, Guangxi Province, China, 27.3.2021, leg. Yi-Wen Liang; ZMB.122758, 4 living-collected specimens, 0.5 to 1.5 m deep on rocks, in Zuo River, Tuozao Village, Jiangzhou District, Chongzuo City, Guangxi Province, China, 27.3.2021, leg. Yi-Wen Liang; ZMB. 122734, 2 living-collected specimens, 0.3 m deep on sand/mud substrate in the wetland near Shitang Village, Mashan County, Nanning City, Guangxi Province, China, 19.3.2022, leg.

Le-Jia Zhang; ZMB.122720, 3 living-collected specimens, 0.5 to 1 m deep on mud substrate, in Bujian River, Yihao Village, Pingguo City, Guangxi Province, China, 19.3.2022, leg. Le-Jia Zhang.

Description

Shell (Fig.13A-Q) medium to large, thin to thick, solid, conical, olive brown to dark brown, with five to six whorls at adulthood, apex acute; teleoconch whorls not inflated, with three smooth main keels, with one to two weaker smooth keel located between suture and the first keel, sometimes one additional weak keel located between first and second/second and third keels, base of shell with one to five weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, orange red or dark purple, outer lip thin and simple, inner lip covered with thick nacreous layers, white or orange red, inner lips of the last growing stages outward folding and tightly overlapping, forming a relatively wide crescent callus-like structure, completely covering umbilicus.

Operculum (Fig.13R-S) corneous, ovate, relatively thick, slightly smaller than shell aperture, dark red; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.15) (n=3) central tooth with one broad central denticle and five to six small sharp cusps on either side; lateral tooth with one broad central denticle and four to five small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and four small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 23 to 24 small sharp cusps.

Remarks

The newly collected specimens from Haikou City of Hainan have a shell that is highly similar to that of the type specimens. *Bakyietaia boettgeri* can be distinguished from most congeners based on its medium-sized to large conical shell with non-inflated whorls, spiral forming a triangle outline and a relatively wide crescent callus-like structure. It can hardly be differentiated from *Bakyietaia duchieri* based on shell morphology, except by its normally non-inflated whorls. The COI tree clearly supports that *B. boettgeri* and *B. duchieri* are distinct species, though. The recommended Chinese name is “伯氏越田螺”.

Habitat and distribution

Mud, sand and rock substrate in rivers, lakes and wetlands of northern Hainan and southern Guangxi Province, China.

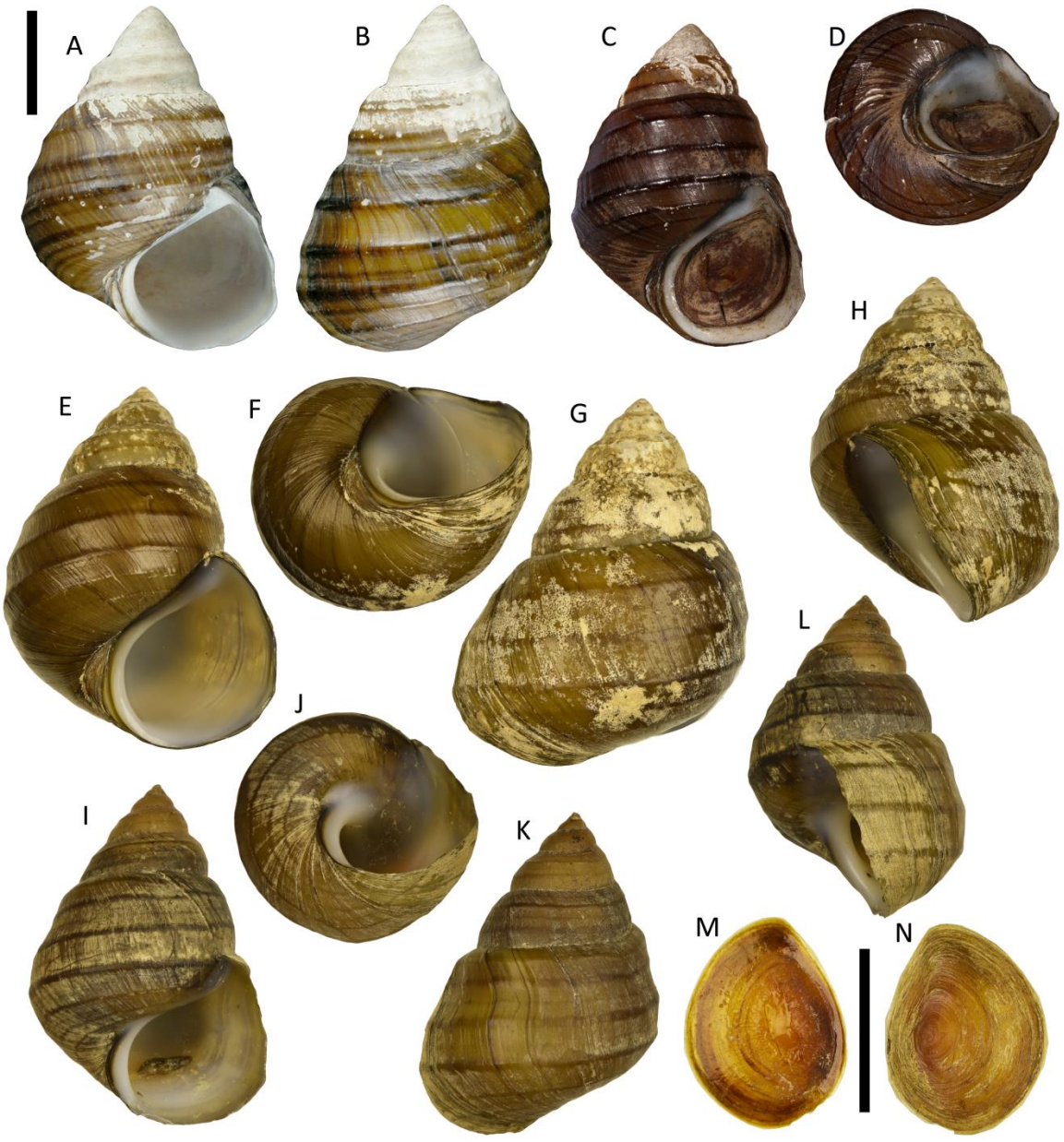


Figure 16. Shells and operculum of *Bakyietaia duchieri*: A-B, holotype, MNHN-IM-2000-4486 (http://coldb.mnhn.fr/catalognumber/mnhn/im/2000-4486, Credit: MNHN - Brabant D. - 2005), Đèo Khách, Lạng Sơn, Vietnam; C-D, SMF.48146, Tonkin, Vietnam; E, ZMB.114431, Cao Bang, Vietnam.

BAKYIETAIA DUCHIERI (FISCHER, 1898) **COMB. NOV.**

Paludina duchieri Fischer, 1898: 330-331, pl. 18 fig. 48-49 ("Riv. Song-KI-Kong, à Déo-Cat", Kỳ Cùng River, at Đèo Khách, Lạng Sơn, Vietnam).

Angulyagra duchieri – Starobogatov, 1970; Thanh *et al*., 2004: 3–4, fig. 5.

Angulyagra boettgeri – Madsen & Hung 2014: 109, figs. 2 (10 left), (10 middle) [non Möllendorf, 1890].

Material examined

Holotype: MNHN-IM-2000-4486, 1803/1/1-2000/12/31, leg. Dr Juillet Billet.

Additional material (Fig.17): *Taia duchieri* SMF.48146, “Tonkin”, Northern Vietnam Slg. O. v. Möllendorff; ZB209-ZB210, 2 living-collected specimens, 0.5 m deep on mud substrate, Shuikou River, near Poliu Village, Shuikou Town, Longzhou County, Chongzuo City, Guangxi Province, China, 4.3.2023.

Description

Shell (Fig.16A-V) large, thick, solid, conical, olive to olive brown, with six whorls at adulthood, apex acute; teleoconch whorls slightly inflated, spiral forming a triangle outline, with three smooth main keels, with one to two weaker smooth keel located between suture and the first keel, , sometimes one additional weak keel located between first and second/second and third keels, base of shell with one to three weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thick nacreous layers, white or orange red, inner lips of the last growing stages outward folding and tightly overlapping, sometimes forming a narrow to wide crescent callus-like structure, this structure or inner lip completely covering umbilicus.

Operculum (Fig.16W-X) corneous, ovate, relatively thick, slightly smaller than shell aperture, dark red; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Remarks

The newly collected specimens in Cao Bang of Vietnam have a shell that is highly similar to that of the type specimens. *Bakyietaia duchieri* is most similar to *Bakyietaia boettgeri* based on shell morphology, except for the normally non-inflated whorls. The distribution areas of these two species are close to each other but not overlapping near the border between Guangxi Province and Vietnam. The recommended Chinese name is “杜氏越田螺”.

Habitat and distribution

Sand substrate and rocks in rivers and lakes of Western Guangxi Province of China and Northern Vietnam.

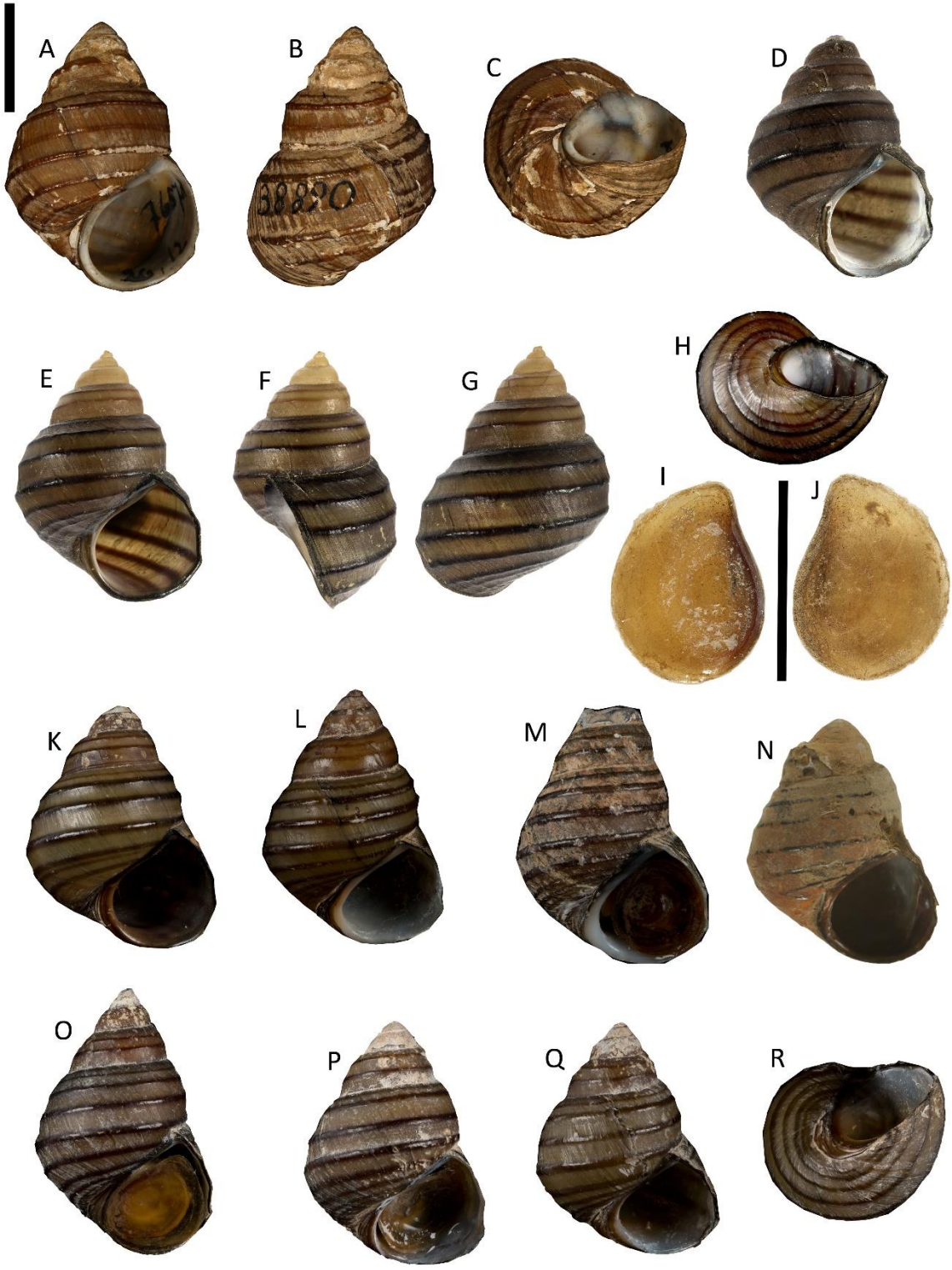


Figure 19. Shells and operculum of *Bakyietaia guangdungensis*: A-C, lectotype, SMF.38890, Guangdong, China; D, ZBMFD.20220225GDGZBYS1, Guangzhou, Guangdong, China; E-H, ZMB.122727-1, Yangshuo, Guangxi, China; I-J, operculum of ZMB.122727-1; K, ZMB.122727-2, Yangshuo, Guangxi, China; L, ZMB.122693-1, Lianzhou, Guangdong,

China; M, ZMB.122768-2, Heshan, Guangxi, China; N, ZMB. 192077, Singapore; O-R, ZMB.122760, Zhaoqing, Guangdong, China; scale bars=1 cm, top left bar applies to all shells, separate bar for operculum I-J.

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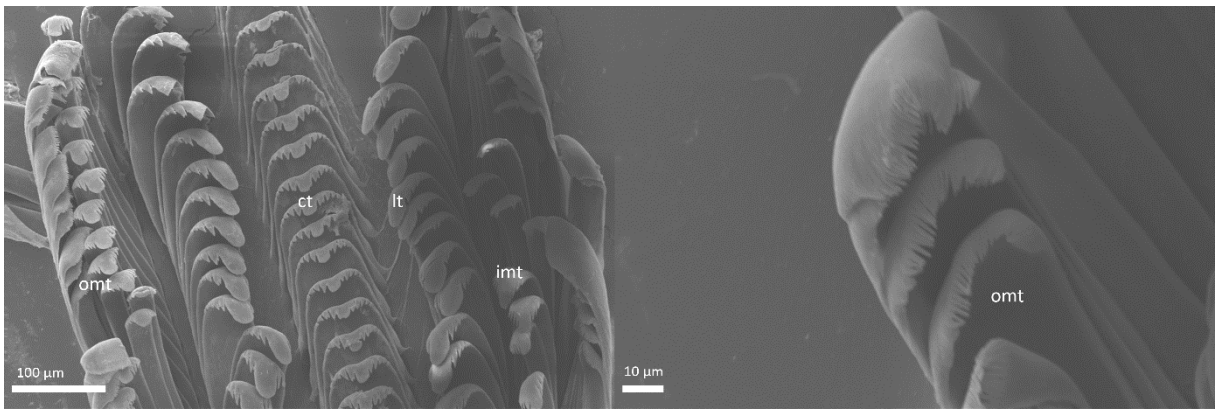


Figure 21. SEM photo of the radula of *Bakyietaia guangdungensis*, ZMB.122701. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA GUANGDUNGENSIS (KOBELT, 1906) **COMB. NOV.**

Vivipara (*quadrata* var.) *heudei* var. *guangdungensis* Kobelt, 1906: 124–125, pl. 21, figs. 12, 14 ("Provinz Guang-dung", Guangdong Province, China.).

Dactylochalamys guangdugensis – Prashad, 1928: 169.

Vivipara polyzonata – Yen, 1939: 37, pl. 3, figs. 14, 15 [non Frauenfeld, 1862].

Angulyagra guangdungensis – Yen, 1943: 128.

Sinotaia polyzonata – Zilch, 1955: 77, fig. 52; Qian *et al*., 2014 [non Frauenfeld, 1862].

Taia polyzonata – Johnson, 1973: 120, 122; Palmieri *et al*., 1980: 40; Ng, 1991: 125; Chan, 1996: 184–185, fig. 2; 1997: 35; Maassen, 2001: 44; Clements *et al*., 2006: 144; Tan & Woo, 2010: 26; Yeo & Chia, 2010: 32; Tan *et al*., 2012: 133 [non Frauenfeld, 1862].

Viviparus polyzonata – Ng *et al*., 1993: 20 [non Frauenfeld, 1862].

Bellamya heudei guangdungensis – Shea, 1994: 3–11.

Sinotaia guangdungensis – Ng *et al*., 2014: 401–408

Angulyagra polyzonata – Madsen & Hung 2014: 110, fig. 2(11) [non Frauenfeld, 1862].

Material examined

Lectotype: SMF.38890, Slg. O. v. Möllendorff, designated by Yen (1939). Paratypes: SMF.38822, 4 dry shells, Slg. O. v. Möllendorff, “China: guang-dung”.

Additional material (Fig.20): ZBMFD.20220225GDGZBYS1, 1 fresh dead specimens, on bank, mud substrate, creeks in Taohuajian Valley, Baiyun Mountain, Guangzhou City, Guangdong Province, China, 25.2.2022, leg. Le-jia Zhang; ZMB.122693A, 1 living-collected specimens, Xian Town, Lianzhou County, Qingyuan City, Guangdong Province, China, 18.11.2019, bought by Hai-Ling Zhang from the local market; ZMB.122727, 4 living-collected specimens, Yangshuo Town, Guilin City, Guangxi Province, China, bought from the local market by Le-Jia Zhang on 14.3.2022; ZMB. 122760, 3 living-collected specimens, 0.1-0.2 m deep under rocks, Qixing Lake, Bohai Park, Zhaoqing City, Guangdong Province, China, 19.1.2020, leg. Ji-Lin Jiang; ZMB., 2 living-collected specimens, 0.5 m deep mud substrate, Huaiji Reservoir, Renyi Village, Heshan County, Laibin City, Guangxi Province, China, 7.1.2020, leg. Ri-Bao Huang; ZMB.192077, 3 living-collected specimens, Canal at Springleaf Rd, Singapore.

Description

Shell (Fig.19I-J) relatively small for the genus, thin but solid, conical, olive to olive brown, with five to six whorls at adulthood, apex acute; teleoconch whorls inflated, sometimes with shoulder, with three smooth main keels, with one to two weaker smooth keel located between suture and the first keel, sometimes one additional weak keel located between first and second/second and third keels, base of shell with three to five weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thick nacreous layers, white or orange, inner lips of the last growing stages outward folding and tightly overlapping, sometimes forming a relatively narrow crescent callus-like structure, this structure or inner lip completely covering umbilicus.

Operculum (Fig.19A-H, K-R) corneous, ovate, relatively thin, slightly smaller than shell aperture, yellow to orange; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.21) (n=3) central tooth with one broad central denticle and four to five small sharp cusps on either side; lateral tooth with one broad central denticle and four small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and three to four small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 28 to 30 small sharp cusps.

Remarks

The newly collected specimens from Guangzhou City of Guangdong have a shell that is highly similar to that of the holotype. *Bakyietaia guangdungensis* can be distinguished from most congeners based on its relatively small conical shell with inflated whorls and a relatively narrow crescent callus-like structure. It largely falls within the shell variation range of *Bakyietaia wilhelmi*, though. However, *B. guangdungensis* has more small cusps on the outer marginal teeth than *B. wilhelmi* (28-29 vs 18-25). DNA barcoding is recommended for the unambiguous identification of these two species. The recommended Chinese name is "广东越田螺".

Habitat and distribution

Mud and rock substrate in rivers and lakes of Western Guangdong and Eastern Guangxi Province, China. Introduced into Singapore and Sydney, Australia, maybe also Malaysia (species in doubt, lack of molecular evidence).

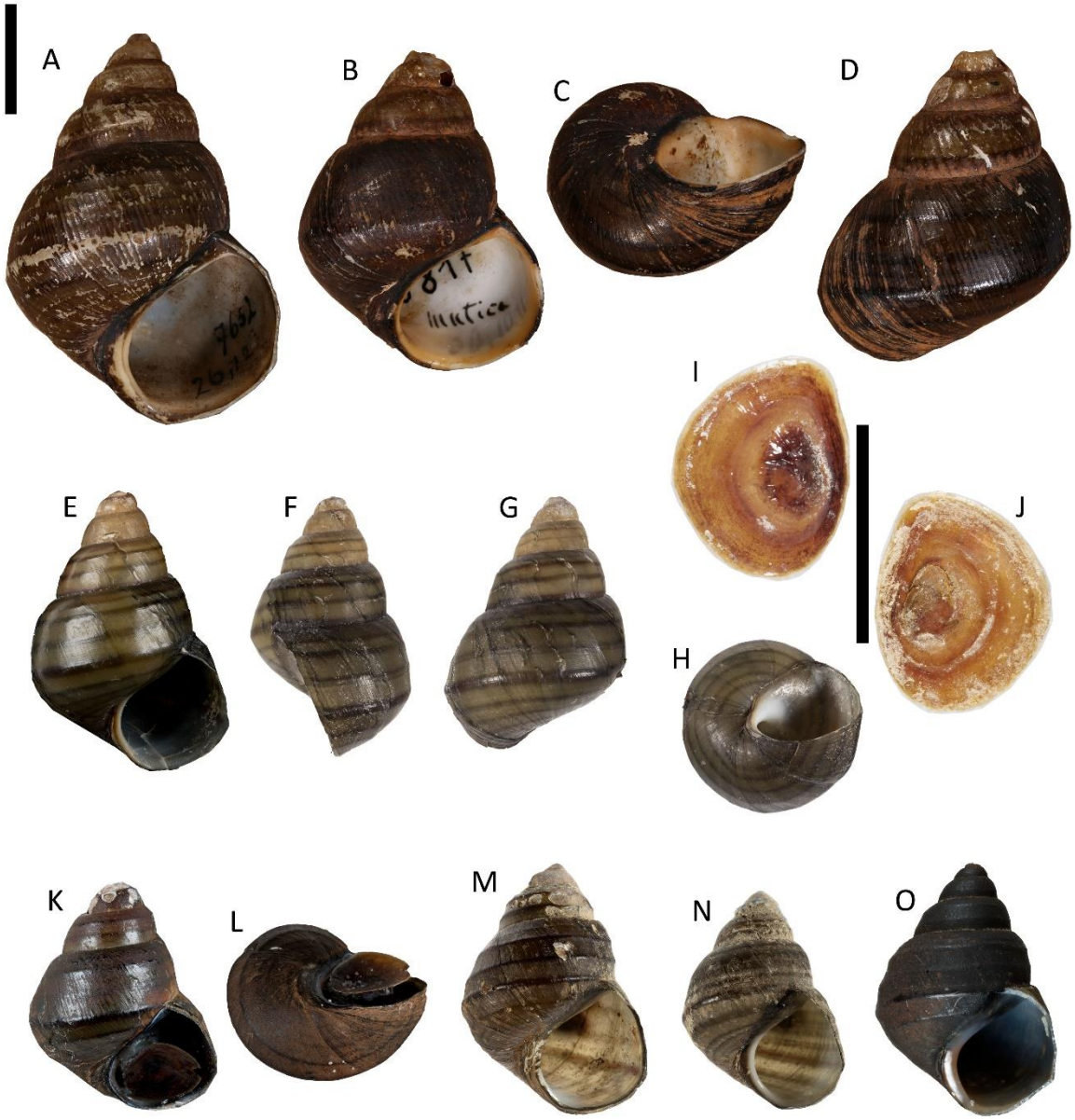


Figure 22. Shells and operculum of *Bakyietaia mutica*: A, paratype SMF.7652, Hainan, China; B-D, holotype, SMF. 38817, Hainan, China; E-H, ZMB.122711-1, Yangshan, Hainan, China; I-J, operculum of ZMB. 122711-1; K-L, ZMB.122752, Yangshan, Hainan, China; M-N, ZMB.122724, Shuikou, Hainan, China; O, ZMB.122746, Xue Village, Hainan, China;

scale bars=1 cm, top left bar applies to all shells, separate bar for operculum I-J.

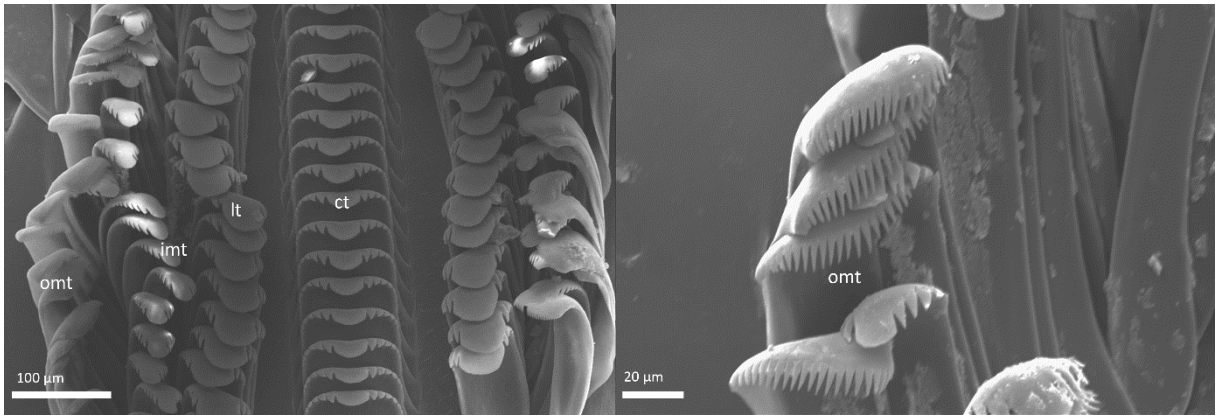


Figure 24. SEM photo of the radula of *Bakyietaia mutica*, ZMB.122752 (left), ZMB.122711 (right). Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA MUTICA (KOBELT, 1909) **COMB. NOV.**

Vivipara boettgeri Kobelt, 1909: 137 [non Heude, 1890, junior homonym].

Vivipara boettgeri mutica Kobelt, 1909: 194, pl. 39, figs. 3–4 ("Hainan", Hainan Province, China).

Viviparus muticus – Yen, 1939: 38, pl. 3, figs. 18–19.

Sinotaia mutica – Zilch, 1955: 77, figs. 50–51.

Angulyagra mutica – Liu *et al*., 1995: 14.

Material examined

Holotype: *Vivipara boettgeri mutica*, SMF.38817, Slg. O. v. Möllendorff; *Vivipara boettgeri*, SMF.7652, Slg. O. v. Möllendorff. Paratypes: SMF.38815, 2 dry shells; SMF.38816, 3 dry shells, "s- China: Insel Hainan";

Additional material (Fig.23): ZMB.122711, 3 living-collected specimens, collected from wetlands near Yangshan Reservoir, Haikou, Hainan Province, China, bought by Sheng-Zhuo Huang from the local market on 20.10.2019; ZMB.122724, 0.5 m deep on wall of cement canal, Shuikou Village, Dingan County, Hainan Province, China, 6.3.2022, leg. Le-Jia Zhang; ZMB.122746, 2 living-collected specimens, 0.1 m deep on rock substrate, wetland in Xue Village, Haikou City, Hainan Province, China, 5.3.2022, leg. Le-Jia Zhang; ZMB.122752, 3 living-collected specimens, 0.1 m deep on rock, a canal of farming field near Yangshan Reservoir, Haikou City, Hainan Province, China, 5.3.2022, leg. Le-Jia Zhang; ZMB.122772, 2 living-collected specimens, 0.5 m deep on rock, a pond in Jinjiahu Village, Wenchang City, Hainan Province, China, 9.11.2020, leg. Ji-Ze Ruan.

Description

Shell (Fig.22A-H, K-O) medium, thin but solid, conical, olive to dark olive brown, with five to six whorls at adulthood, apex acute; teleoconch whorls inflated, sometimes with shoulder, spiral always forming a triangle outline, with three weak but obvious smooth main keels, with one to two even weaker smooth keel located between suture and the first keel, sometimes one additional weak keel located between first and second/second and third keels, base of shell with two to four weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thick nacreous layers, always white, inner lips of the last growing stages outward folding and tightly overlapping, sometimes forming a relatively wide crescent callus-like structure, this structure or inner lip completely covering umbilicus.

Operculum (Fig.22I-J) corneous, ovate, relatively thin, slightly smaller than shell aperture, dark orange to red; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.24) (n=3) central tooth with one broad central denticle and four to five small sharp cusps on either side; lateral tooth with one broad central denticle and four to five small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and five to six small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 19 to 24 small sharp cusps.

Remarks

The newly collected specimens from Haikou City of Hainan have a shell that is highly similar to that of the holotype. *Bakyietaia mutica* is one of the "weak main keels" *Bakyietaia* species. It can be easily distinguished from most congeners based on its medium-sized thin conical shell with three very weak main keels. It is morphologically similar to *Bakyietaia fontinalis* and *Bakyietaia naiadica* However, it can be distinguished from *B. fontinalis* based on the inflated whorls (vs non-inflated whorls in *B. fontinalis*) and less cusps on outer marginal teeth (19 to 24 vs 26 to 29); it can be distinguished from *B. naiadica* based on its triangle outline of shell (vs more cylindrical outline of shell in *B. naiadica*).

The recommended Chinese name is "钝形越田螺".

Habitat and distribution

Rock substrate in rivers and lakes of northern Hainan Province, China.


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Figure 25. Shells and operculum of *Bakyietaia wilhelmi*: A-C, holotype, SMF.40812, Guangdong, China; D, ZMB.122686-2, Jiangxikong, Guangdong, China; E, ZMB.122696, Heyuan, Guangdong, China; F-I, ZMB.122686-1, Jiangxikong, Guangdong, China; J, ZMB.122689, Fogang, Guangdong, China; K-L, ZMB.122688, Zhuhai, Guangdong, China; M, ZMB.122692, Shenzhen, Guangdong, China; N-O, ZMB.122729, Gejiu, Yunnan, China; P-Q, ZMB.122685, Liuzhou, Guangxi, China; R, ZMB.122751, Zhanjiang City, Guangdong, China; S, ZMB.122737-3, Ledong, Hainan, China; T, ZMB.122709, Yangshan, Hainan, China; U, ZMB.114206, Ha Quang, Vietnam; V, ZMB.122770, Guigang, Guangxi, China; W, 122693-2, Lianzhou, Guangdong, China; X, ZMB.122760-1, Zhaoqing, Guangdong, China; Y, ZMB.122768-1, Heshan, Guangxi, China; Z, ZMB.122704, Jieyang, Guangdong, China; AA-AB, operculum of ZMB.122686-1; AC-AD, operculum of ZMB.114206; AE-AF, operculum of ZMB.122685; scale bars=1 cm, top left bar applies to all shells, separate bar for opercula AA-AF.

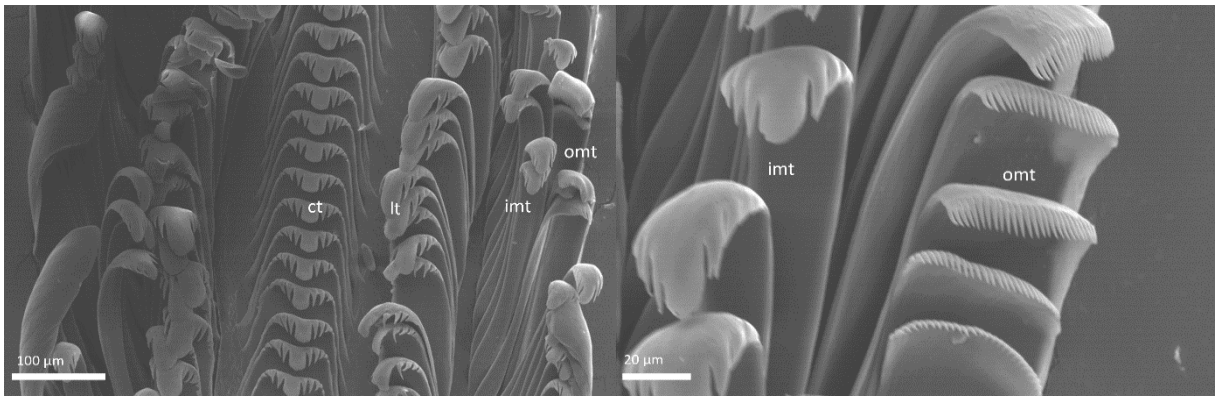


Figure 27. SEM photo of the radula of *Bakyietaia wilhelmi*, ZMB. 122686. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA WILHELMI (YEN, 1939) **COMB. NOV.**

Viviparus wilhelmi Yen, 1939: 37, pl. 3, fig. 16 ("Kanton", Guangzhou City, Guangdong, China).

Sinotaia wilhelmi – Zilch, 1955: 77, figs. 56.

Angulyagra wilhelmi – Starobogatov, 1970: 202; Liu *et al*., 1995: 14.

Angulyagra boettgeri – Thanh *et al*., 2014: 3–4, fig. 5 [non Möllendorf, 1890].

Sinotaia guangdungensis – Ng *et al*., 2017 [non Kobelt, 1906].

Material examined

Holotype: *Taia wilhelmi*, SMF.40812, Slg. O. v. Möllendorff. Paratypes: *Taia wilhelmi*, SMF.40243, 18 specimens, "Canton", Slg. O. v. Möllendorff.

Additional material (Fig.26): ZMB.122768A, 1 living-collected specimens, 0.5 m deep mud substrate, Huaiji Reservoir, Renyi Village, Heshan County, Laibin City, Guangxi Province, China, 7.1.2020, leg. Ri-Bao Huang; ZMB.122709, 2 living-collected specimens, collected from wetlands near Yangshan Reservoir, Haikou, Hainan Province, China, bought by Sheng-Zhuo Huang from the local market on 20.10.2019; ZMB.122771, 2 living-collected specimens, 0.14 m deep on mud or rock substrate of creeks, Liuzu Town, Xinxing County, Yunfu City, Guangdong Province, China, 4.1.2020; Coll/ Fu-Ying Liang; ; ZMB.122760-1, 1 living-collected specimens, 0.1-0.2 m deep under rocks, Qixing Lake, Bohai Park, Zhaoqing City, Guangdong Province, China, 10.1.2020, leg. Ji-Lin Jiang; ZMB.122769, 2 living-collected specimens, East Lake, Donghu Park, Chengdu City, Sichuan Province, China, 12.10.2022/10/12; ZMB.122688, 4 living-collected specimens, 0.1-0.2 m deep on mud substate, a canal in Nanxin, Pingsha Town, Zhuhai City, Guangdong Province, China, 12.10.2022; MZ596234, Malaysia, Sabah; ZMB. 122770, 2 living-collected specimens, 0.5 m deep on mud substrate, Niuyun Reservoir, Gangbei District, Guigang City, Guangxi Province, China, 7.1.2020, leg. Ri-Bao Huang; LJZ.22MZ, 4 dry shells in a canal, Sanhe Town, Dapu County, Meizhou City, Guangdong Province, China, 2.2.2022, leg. Le-Jia Zhang; ZMB.122737-3, 1 living-collected specimens, 0.1 m deep sand substrate, in Honghu Lake, Honghu Village, Ledong Li Autonomous County, Hainan Province, China, 9.3.2022, leg. Le-Jia Zhang; ZMB.122729, 4 living-collected specimens, 0.1 m deep canal, in Zhadian Village, Gejiu City, Yunnan Province, China, 21.3.2022, leg. Le-Jia Zhang; ZMB.122751, 3 living-collected specimens, 2 m deep on mud substrate, a pond in Zhiliao Village, Zhanjiang City, Guangdong Province, China, 3.3.2022, leg. Le-Jia Zhang; ZMB.122689, 3 living-collected specimens, 0.5 m deep on mud or rock substrate, Longxi River, under Longxi Bridge, Lianxi Village, Shijiao Town, Fogang County, Qingyuan City, Guangdong Province, China, 6.12.2019, leg. Chang-Le Huang; ZMB.122700, 3 living-collected specimens, 0.1-0.25 m deep on rock, Xiaodu River, Changxing Village, Wuhua County, Meizhou City, Guangdong Province, China, 28.11.2019, leg. Yu-Hui Cai; ZMB.122685, 2 living-collected specimens, 0.5 m deep on mud substrate, canal near lotus fields, Hepingtun, Baipeng Village, Liujiang District, Liuzhou City, Guangxi Province, China, 19.10.2019, leg. Ping Zeng; ZMB.122693B, 1 living-collected specimens, Xian Town, Lianzhou County, Qingyuan City, Guangdong Province, China, 18.11.2019, bought by Hai-Ling Zhang from the local market; ZMB.122696, 4 living-collected specimens, 0.4 m deep in pond, Xinfengjiang Reservoir, Dongyuan County, Heyuan City, Guangdong Province, China, 18.11.2019, leg. Chun-Di Chen; ZMB.122702, 4 living-collected specimens, 1-2 m deep on mud substrate in fish pond, Laozhutang, Tianxin Village, Xintian Town, Luhe County, Shanwei City, Guangdong Province, China, 15.12.2019, leg. Yuan-Shan Qiu; ZMB.122699, 4 living-collected specimens, 0.2 m deep on mud or rock substrate, Mojiangping Lake, Diba, Taiping Town, Shixing County, Shaoguan City, Guangdong Province, China, 5.12.2019, leg. Yong-Ming Nie; ZMB.122704, 4 living-collected specimens, 4-5 m deep in creek, Xiashanweijiao, Nanxi Town, Puning County,

Jieyang City, Guangdong Province, China, 4.12.2019, leg. Mei Yang; ZMB.122686, 4 living-collected specimens, 0.4 m deep on sand and rock substrate, Jiangxikong, Yangshan County, Qingyuan City, Guangdong Province, China, 4.12.2019, leg. Jian-Guo Chen; ZMB.122695, 2 living-collected specimens, 0.5 m deep on mud substrate in creek, Yakoukan Village, Lingshan, Zengcheng District, Guangzhou City, Guangdong Province, China, 4.12.2019, leg. Da-Ming Shi; ZMB.122692, 2 living-collected specimens, 0.5 m deep on mud substrate, outlet of a reservoir near Dawang Cemetery, Baoan District, Shenzhen City, Guangdong Province, China, 18.12.2019, leg. Fan Qiu; ZMB.122723-2, 1 living-collected specimens, 0.1 m deep on sand or rock substrate, creek near Nawang Village, Baise City, Guangxi Province, China, 20.3.2022, leg. Le-Jia Zhang; ZMB.114206, 4 living-collected specimens, Ha Quang River near Pac Bo at Chinese border, Cao Bang, Vietnam, 21.3.2006, bought by Frank Köhler from the local market; ZMB.114202, 4 living-collected specimens, Bang River, Cao Bang, Vietnam, 21.3.2006, leg. Frank Köhler. MZ596234.1 Sabah Malaysia.

Description

Shell (Fig.25A-Z) small to medium, thin to thick, solid, conical, olive to dark olive brown, with five to six whorls at adulthood, apex acute; teleoconch whorls sometimes without shoulder and spiral forming a triangle outline, sometimes inflated and with shoulder, with three smooth main keels, with one to two weaker smooth keel located between suture and the first keel, , sometimes one additional weak keel located between first and second/second and third keels, base of shell with two to five weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thick nacreous layers, white or orange, inner lips of the last growing stages outward folding and tightly overlapping, sometimes forming a rather wide crescent callus-like structure, this structure or inner lip completely covering umbilicus.

Operculum (Fig.25AA-AF) corneous, ovate, relatively thin, slightly smaller than shell aperture, yellow to orange; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.27) (n=6) central tooth with one broad central denticle and four to five small sharp cusps on either side; lateral tooth with one broad central denticle and four small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and three to five small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 18 to 25 small sharp cusps.

Remarks

The newly collected specimens from Qingyuan City of Guangdong have a shell that is highly similar to that of the holotype. *Bakyietaia wilhelmi* is the most common species of this genus. It has the widest range of distribution, and displays an extremely high polymorphism of shell characters. Besides, this species was found sympatrically with other *Bakyietaia* species (*B. boettgeri*, *B. duchieri* and *B. guangdungensis*) in some localities (such as Qixing Lake in Zhaoqing City of Guangdong, Honghu Lake in Ledong County of Hainan). The morphotype that is similar to the holotype can be can be distinguished from most congeners based on its relatively small conical shell with non-inflated whorls and a relatively wide crescent callus-like structure. However, some morphotypes cannot be distinguished from *Bakyietaia guangdungensis* based on their shell morphology. The COI tree supports the status of *B. wilhelmi* and *B. guangdungensis* as distinct species, which are not even closely related. In addition, *B. wilhelmi* always has fewer small cusps on the outer marginal teeth than *B. guangdungensis* (18-25 vs 28-30). DNA barcoding is considered necessary to unambiguously differentiate *B. wilhelmi* and *B. guangdungensis*. The recommended Chinese name is “威廉越田螺”.

Habitat and distribution

Mud, sand and rock substrate in rivers, wetlands and lakes of Guangdong, Hainan, Guangxi and southern Yunnan Province, China and northern Vietnam. Introduced into Chengdu City, Sichuan Province, China and northern Borneo, Malaysia.

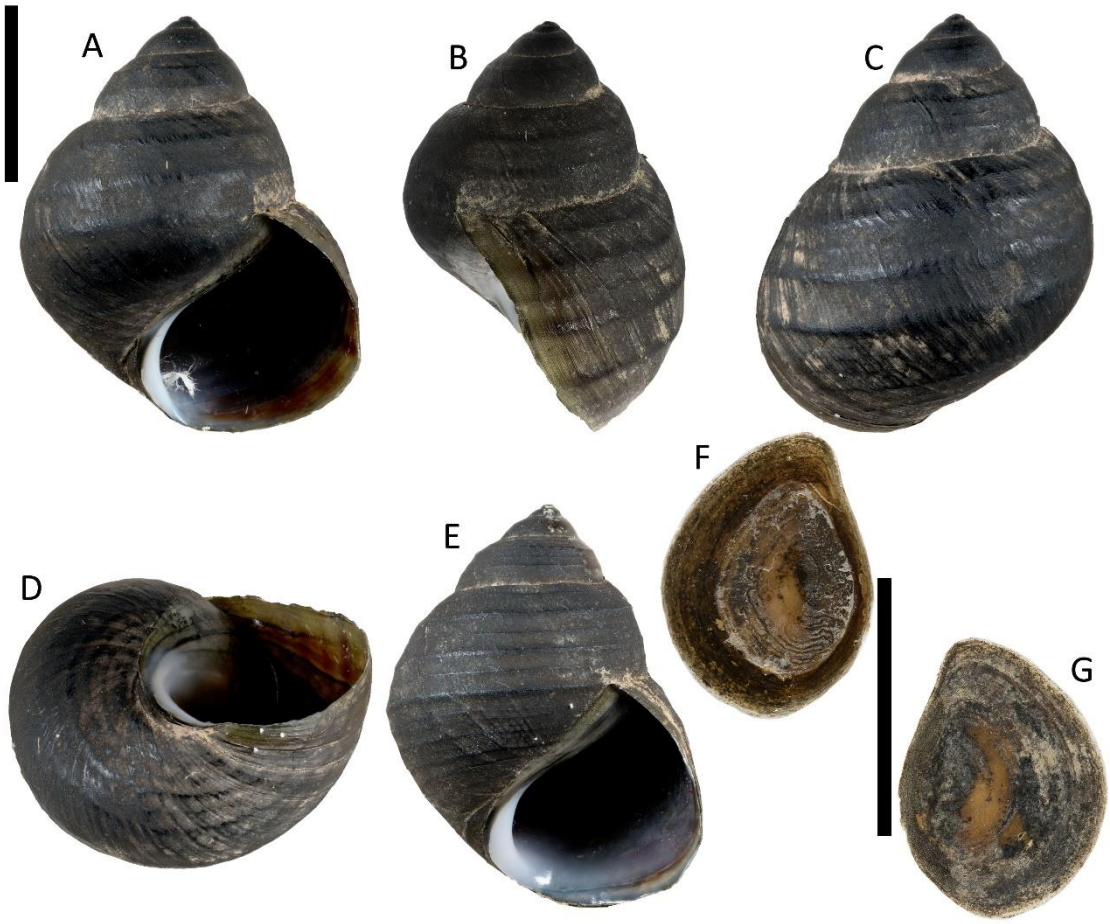


Figure 28. Shells and operculum of *Bakyietaia chenghuang*: A-D, holotype, ZBMFD.20220312GXYKCH1, Chenghuang Town, Guangxi, China; E, paratype, ZMB.122722, Chenghuang Town, Guangxi, China; F-G, operculum of ZBMFD.20220312GXYKCH1; scale bars=1 cm, top left bar applies to all shells, separate bar for operculum F-G.

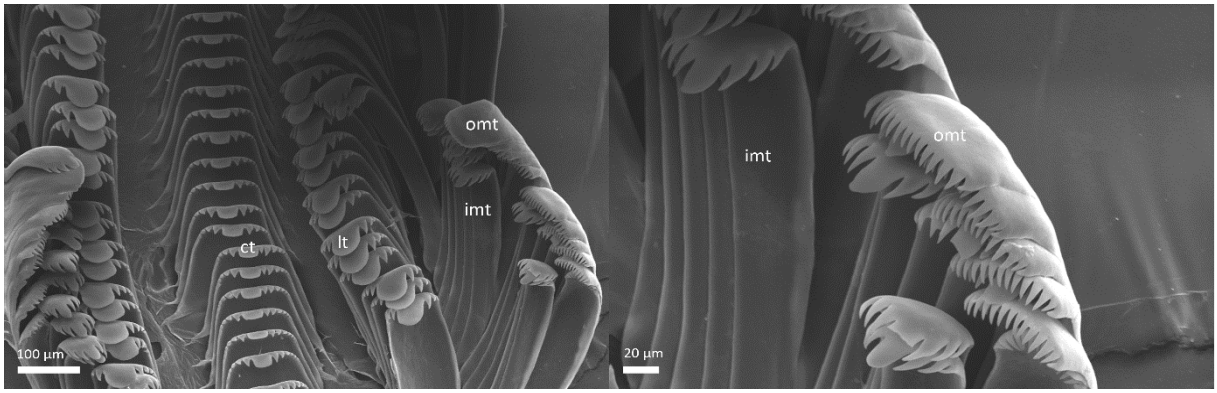


Figure 30. SEM photo of the radula of *Bakyietaia chenghuang*, ZMB. 122722. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA CHENGHUANG ZHANG & YEN **SP. NOV.**

Etymology: "Cheng Huang" means "city god" in Chinese, referring to the type locality in Chenghuang Town. Formed as a noun in apposition. The recommended Chinese name is "城隍越田螺".

Material examined

Holotype: ZBMFD.20220312GXYKCH1, living collected specimen, 0.5 m deep on rock, a river near Shirentou Mountain, Chenghuang Town, Yulin City, Guangxi Province, China, 13.3.2022, leg. Le-Jia Zhang. Paratypes: 10 living collected specimens, same collecting data as the holotype, 4 kept in ZBMFD (ZBMFD.20220312GXYKCH2), 2 kept in ZMB (ZMB.122722), 4 kept in ZLJ (22YLCH) (Fig.29).

Description

Shell (Fig.28A-E) medium, thin but solid, broadly conical or subglobose, olive to olive brown, with five whorls at adulthood, apex acute; teleoconch whorls inflated, with three smooth main keels, with one to two weaker smooth keel located between suture and the first keel, sometimes one additional weak keel located between first and second/second and third keels, base of shell with three to five weak keels; aperture ovate, large, around half of shell in height, inner shell surface bluish white to purple white, outer lip thin and simple, inner lip covered with thick nacreous layers, always white, inner lips of the last growing stages outward folding and tightly overlapping, forming a very narrow crescent callus-like structure or without this structure, inner lip or this structure completely covering umbilicus.

Operculum (Fig.28F-G) corneous, ovate, thin, obviously smaller than shell aperture, yellow; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins.

Radular (Fig.30) (n=2) central tooth with one broad central denticle and four to five small sharp cusps on either side; lateral tooth with one broad central denticle and four small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and three small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 15 to 16 small sharp cusps.

Remarks

Bakyietaia chenghuang can be easily distinguished from most congeners based on its subglobose thin shell with a wide aperture and a very small operculum. It can be

differentiated from the morphologically similar species *Bakyietaia subcostata* based on much fewer cusps on the outer marginal teeth (15-16 vs 29-43).

Habitat and distribution

Only known from the rock substrate of the type locality in Guangxi Province, China.

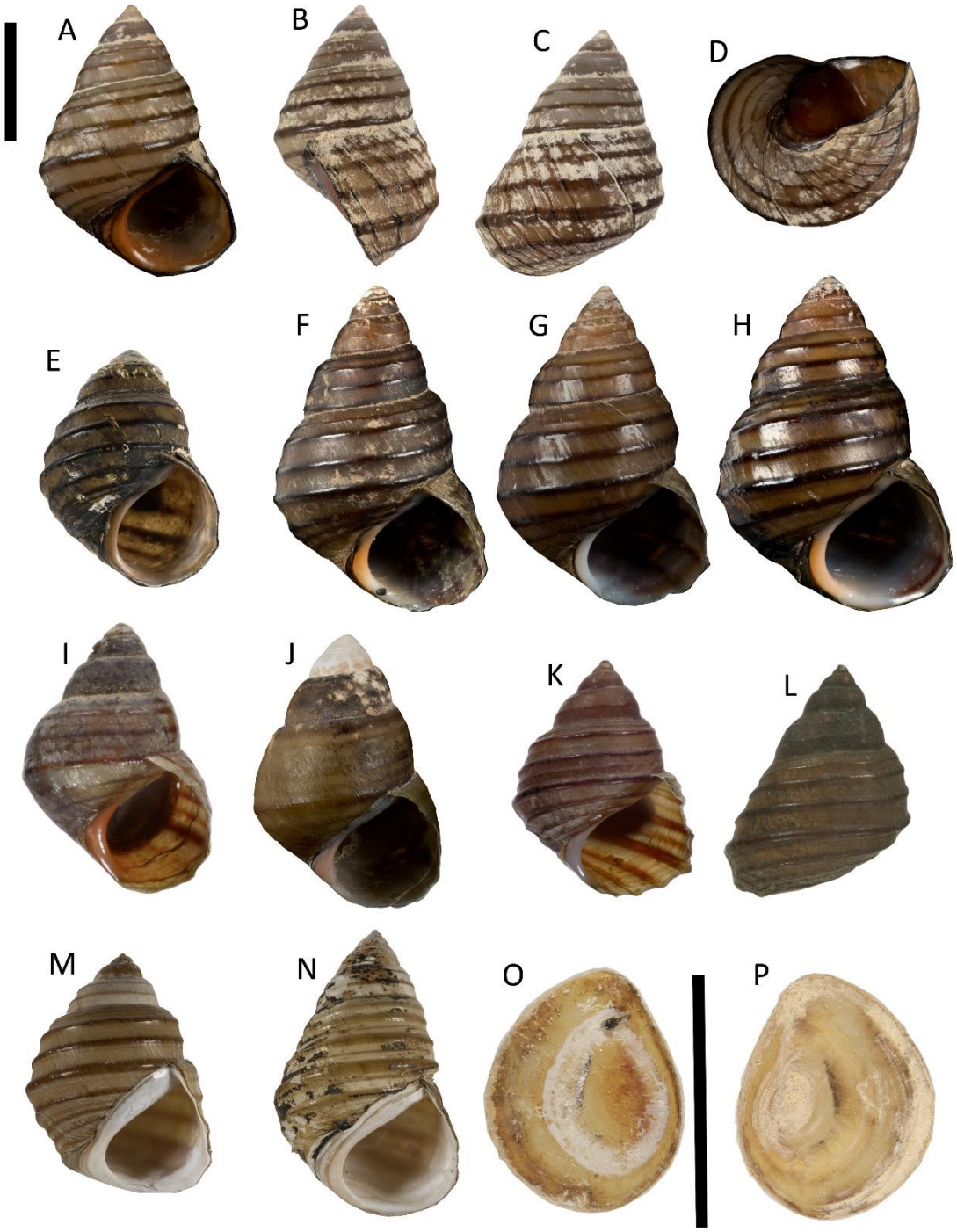


Figure 16. Shells and operculum of *Bakyietaia jingweiae*: A-D, holotype, ZBMFDB.20191230GXJXLT1, Jingxi, Guangxi, China; E, ZBMFDB.20220320GXBSNW1,

Nawang, Guangxi, China; F-H, ZBMFDB.20220309HNLDHH1, Ledong, Hainan, China; I, ZMB.114407 Nghê An, Vietnam; J, ZBMFDB.20220309HNCHSJ1, Changhua, Hainan, China; K-L, ZMB.114189, Ba Be Lakes, Bac Kan, Vietnam; M-N, ZMB.114430, Ba Be Lakes, Bac Kan, Vietnam; O-P, operculum of ZMB.122701; scale bars=1 cm, top left bar applies to all shells, separate bar for operculum O-P.

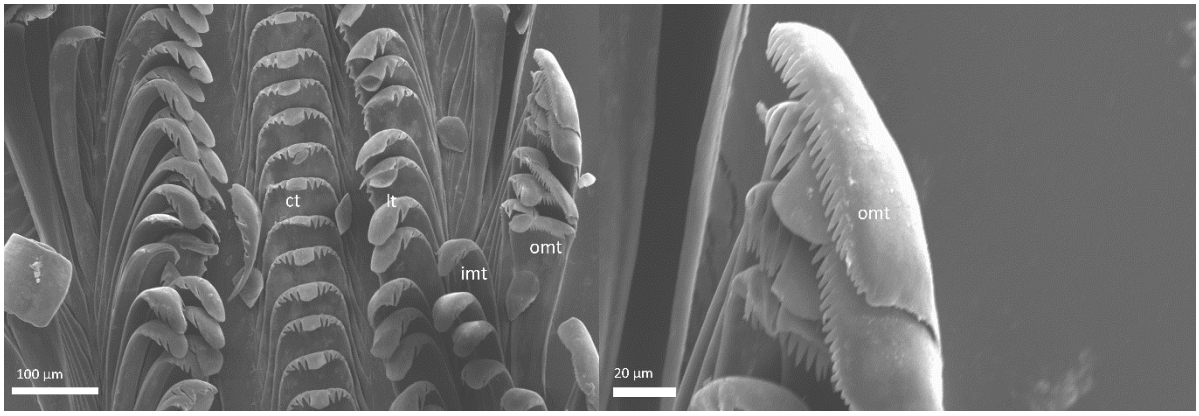


Figure 18. SEM photo of the radula of *Bakyietaia jingweiae*, ZMB.122701. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA JINGWEIAE YEN & ZHANG **SP. NOV.**

Etymology: Named after "Jingwei", a mythical bird of China trying to fill up the sea, which was transformed from the Flame Emperor Yandi's daughter Nüwa, who drowned in the sea. The recommended Chinese name is "精卫越田螺".

Material examined

Holotype: MNHN-IM-2000-4486, 1803/1/1-2000/12/31, leg. Dr Juillet Billet.

Additional material (Fig.17): *Taia duchieri* SMF.48146, "Tonkin", Northern Vietnam Slg. O. v. Möllendorff; ZMB. 114431, 4 living-collected specimens, river Sông Bằng, Cao Bang, Vietnam, year 2000, leg. IEBR; ZMB.114407, 3 living-collected specimens, Ca River drainage, Ho-Chi-Minh-Highway between Yen Cat and Thái Hòa, Nghê An, Vietnam, 21-31.10.2006, leg. Frank Köhler; ZMB.114430, 2 living-collected specimens, Ba Be Lakes, Bac Kan, Vietnam, year 2001, leg. IEBR; ZMB.114189, 10 living-collected specimens, ferry port, Ba Be Lakes, Bac Kan, Vietnam, 21.3.2006, leg. Frank Köhler; ZMB.122701, 4 living-collected specimens, 0.1 m on rock, Longtan Wetland, Xinjing Town, Jingxi County, Baise City, Guangxi Province, China, 30.12.2019, leg. Hua-Pu Huang; ZBMFDB.20220320GXBSNW1, 1 living-collected specimens, 0.1 m deep on sand or rock substrate, creek near Nawang Village, Baise City, Guangxi Province, China, 20.3.2022, leg.

Le-Jia Zhang; ZMB.122737-1/-2/-4, 3 living-collected specimens, 0.1 m deep sand substrate, in Honghu Lake, Honghu Village, Ledong Li Autonomous County, Hainan Province, China, 9.3.2022, leg. Le-Jia Zhang; ZBMFDB.20220309HNCHSJ1, 1 living-collected specimens, 0.5 m deep on rock, in Changhua River, Sanjia Village, Changhua Town, Changjiang Li Autonomous County, Hainan Province, China, 2022/3/9, leg. Le-Jia Zhang.

Description

Shell (Fig.16A-V) small to medium, thin to thick, solid, conical, olive to olive brown, with five to six whorls at adulthood, apex acute; teleoconch whorls slightly inflated, spiral forming a triangle outline, with three smooth main keels, with one to two weaker smooth keel located between suture and the first keel, , sometimes one additional weak keel located between first and second/second and third keels, base of shell with one to three weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thick nacreous layers, white or orange red, inner lips of the last growing stages outward folding and tightly overlapping, sometimes forming a narrow to wide crescent callus-like structure, this structure or inner lip completely covering umbilicus.

Operculum (Fig.16W-X) corneous, ovate, relatively thick, slightly smaller than shell aperture, dark red; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.18) (n=4) central tooth with one broad central denticle and four to six small sharp cusps on either side; lateral tooth with one broad central denticle and five to six small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and four to five small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 26 to 28 small sharp cusps.

Remarks

The newly collected specimens in Cao Bang of Vietnam have a shell that is highly similar to that of the type specimens. *Bakyietaia duchieri* is most similar to *Bakyietaia boettgeri* based on shell morphology, except for the normally non-inflated whorls. The distribution areas of these two species are close to each other but not overlapping near the border between Guangxi Province and Vietnam. The recommended Chinese name is “杜氏越田螺”.

Habitat and distribution

Sand substrate and rocks in rivers and lakes of Western Guangxi and Western Hainan Province, China and Northern Vietnam.

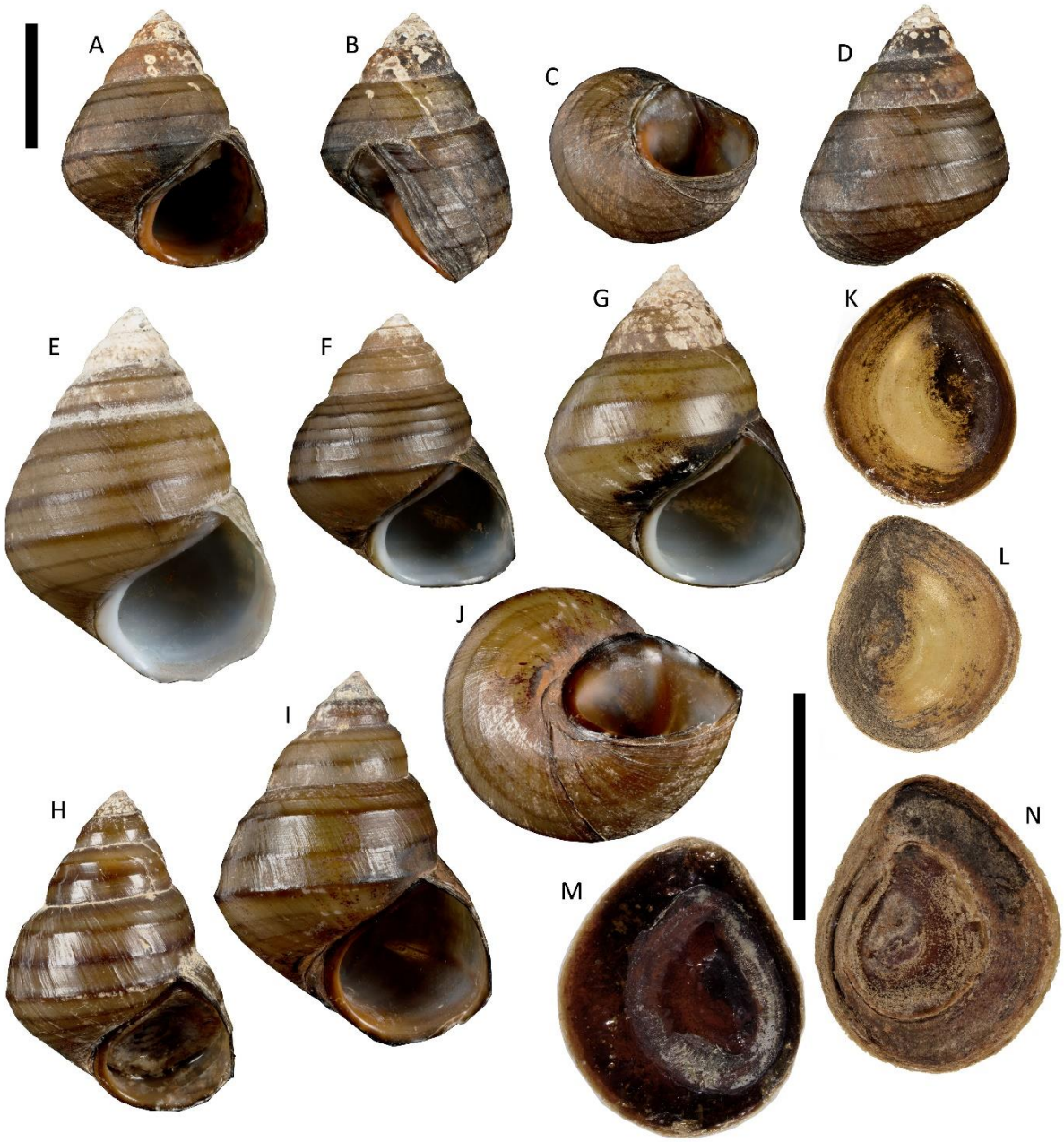


Figure 31. Shells and operculum of *Bakyietaia liusanjieae*: A-D, holotype, ZBMFD.20220316GXHCXH1, Xinhui, Guangxi, China; E, paratype, ZLJ.22HCLSJ, Liusanjie Town, Guangxi, China; F-G, paratypes, ZBMFD.20220316GXHCXH2, Xinhui, Guangxi, China; K-L, operculum of ZBMFD.20220316GXHCXH2; H, paratype, ZMB.122763, Hezhou, Guangxi, China; I-J, paratype, ZMB.122722, Baishou, Guangxi, China; M-N, operculum of ZMB.122722; scale bars=1 cm, top left bar applies to all shells, separate bar for opercula K-N.

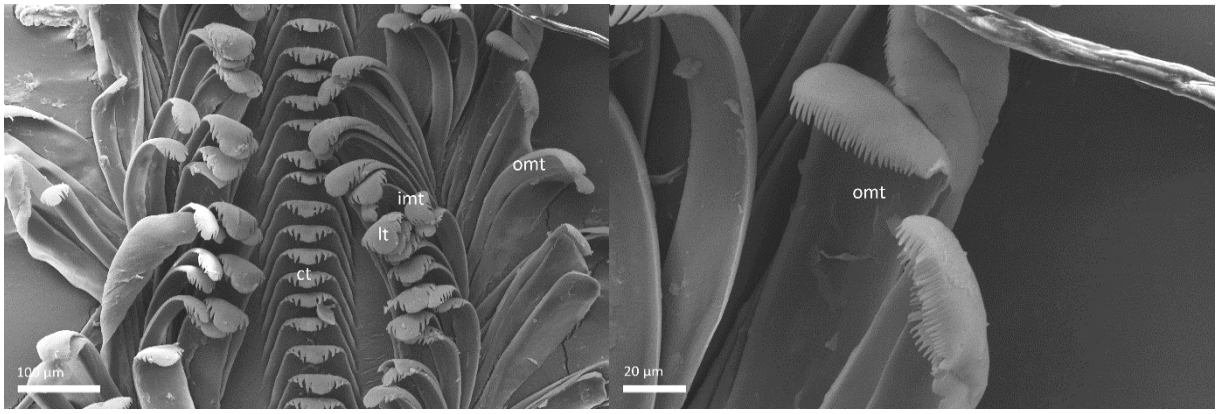


Figure 33. SEM photo of the radula of *Bakyietaia liusanjieae*, ZMB. 122722. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA LIUSANJIEAE ZHANG & YEN **SP. NOV.**

Etymology: Named after "Liu Sanjie" (刘三姐, Liu family's third daughter), a legendary folk music singer of the Zhuang people in Guangxi. The type locality of this species is close to her hometown. The recommended Chinese name is "歌仙越田螺".

Material examined

Holotype: ZBMFD.20220316GXHCXH1, living collected specimen, 0.5 m deep on dam, a river in Xinhui Village, near Hemu Town, Luocheng Mulao Autonomous County, Hechi City, Guangxi Province, China, 16.3.2022, leg. Le-Jia Zhang. Paratypes: 6 living collected specimens, same collecting data as the holotype, 2 kept in ZBMFD (ZBMFD.20220316GXHCXH2), 2 kept in ZMB (ZMB.122722), 2 kept in ZLJ (22HCXH); 5 living collected specimens, 0.5 m deep on mud and sand substrate, Baishou River, Baishou Town, Guilin City, Guangxi Province, China, 15.3.2022, leg. Le-Jia Zhang, 2 kept in ZBMFD (ZBMFD.20220315GXGLBS1), 2 kept in ZMB (ZMB.122738), 1 kept in ZLJ (22GLBS); 1 fresh dead shell with operculum, on the bank of Xiajian River, Liusanjie Town, Yizhou District, Hechi City, Guangxi Province, China, 16.3.2022, leg. Le-Jia Zhang, kept in ZLJ (22HCLSJ); 2 dry shells collected on the bank of Long River, Yizhou District, Hechi City, Guangxi Province, China, 17.3.2022, leg. Le-Jia Zhang, kept in ZLJ; 5 living collected specimens, 0.5 m deep on mud substrate of a pond, Shangpu, Fengzhen Village, Tonggu Town, Zhongshan County, Hezhou City, Guangxi Province, China, 9.3.2020, leg. Shui-Yang Huang, , 2 kept in ZBMFD (ZBMFD.20200309GXHZSP1), 1 kept in ZMB (ZMB. 122763), 2 kept in ZLJ (20HZ) (Fig.32).

Description

Shell (Fig.31A-J) medium, thin but solid, broadly conical to conical, olive to olive brown, with five to six whorls at adulthood, apex acute; teleoconch whorls not inflated, spiral forming a triangle outline, with three smooth relatively weak main keels, with one to two

even weaker smooth keel located between suture and the first keel, sometimes one additional weak keel located between first and second/second and third keels, base of shell with one to five weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, orange red or dark purple, outer lip thin and simple, inner lip covered with thick nacreous layers, white or orange red, inner lips of the last growing stages outward folding and tightly overlapping, sometimes forming a relatively wide crescent callus-like structure, this structure or inner lip completely covering umbilicus.

Operculum (Fig.31K-N) corneous, ovate, relatively thick, mostly slightly smaller than shell aperture, sometimes obviously smaller than shell aperture, dark orange to dark red; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.33) (n=3) central tooth with one broad central denticle and three to four small sharp cusps on either side; lateral tooth with one broad central denticle and two to three small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and five small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 26 to 27 small sharp cusps.

Remarks

Bakyietaia liusanjieae can be distinguished from its congeners, especially *Bakyietaia boettgeri*, based on its medium-sized to large shell with non-inflated whorls and the depressed triangle outline of shell and relatively weaker main keels. The distribution areas of of *B. liusanjieae* and *B. boettgeri* are not overlapping (Northern Guangxi vs Southern Guangxi).

Habitat and distribution

Rock substrate in rivers of Northern Guangxi Province, China.

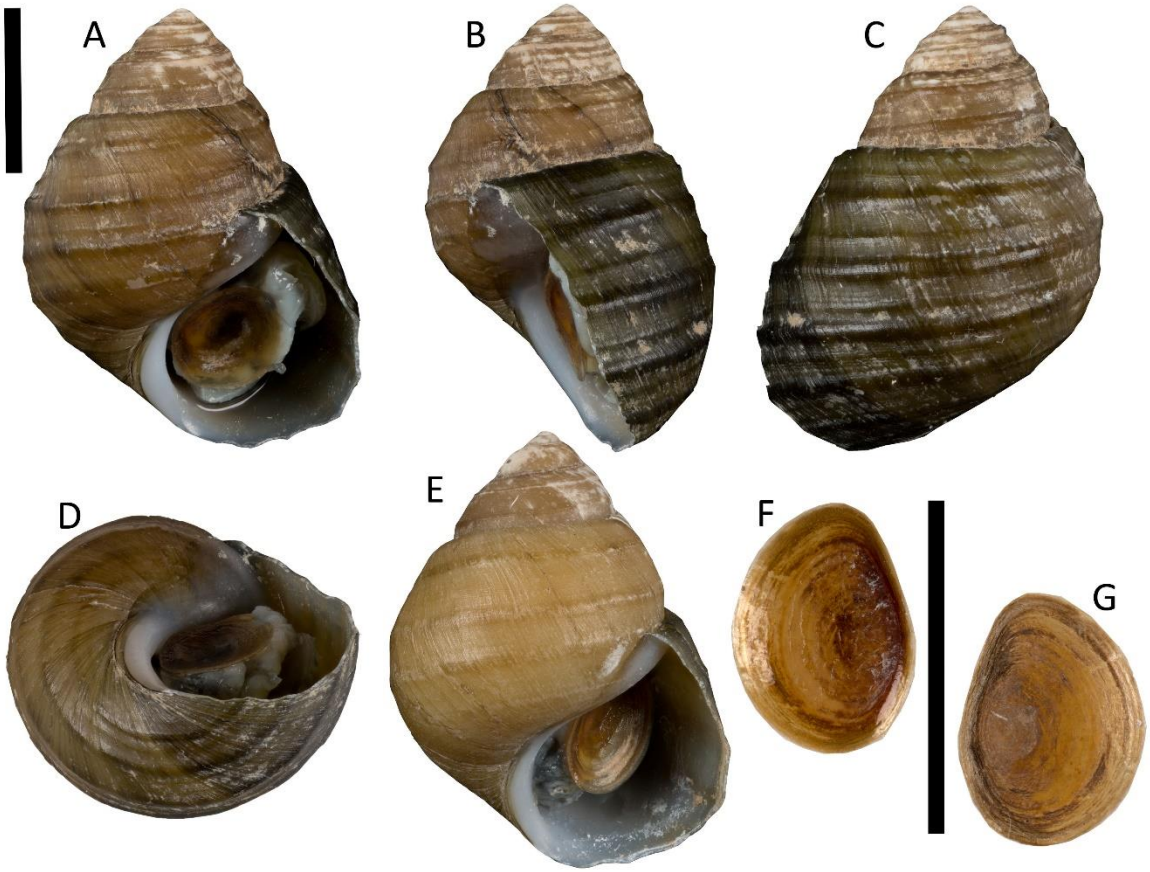


Figure 34. Shells and operculum of *Bakyietaia paradoxa*: A-D, holotype, ZBMFD.20220900GXBSSL1, Silin, Guangxi, China; E, paratype, ZMB.20220900GXBSSL2, Silin, Guangxi, China; F-G, operculum of ZBMFD.20220900GXBSSL2; scale bars=1 cm, top left bar applies to all shells, separate bar for operculum F-G.

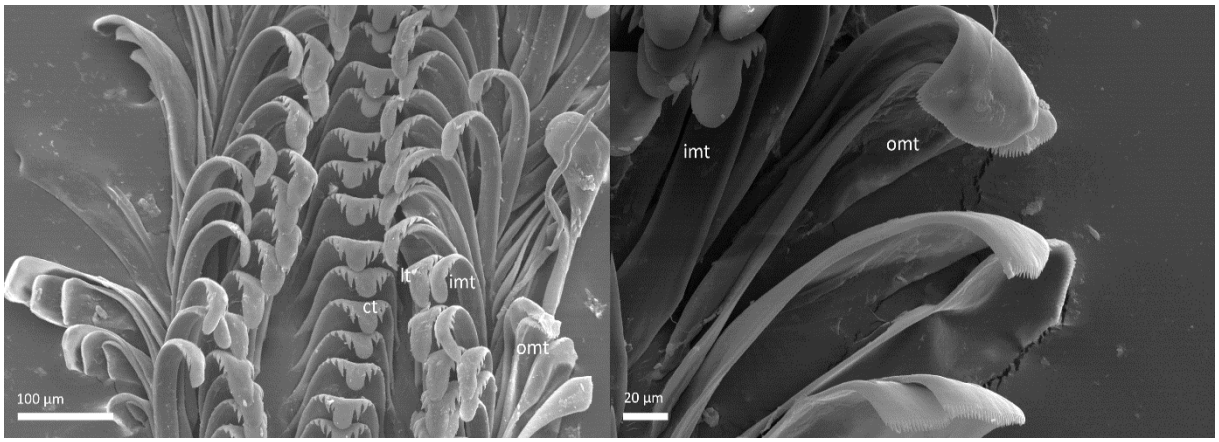


Figure 36. SEM photo of the radula of *Bakyietaia paradoxa*, ZMB.. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA PARADOXA ZHANG & YEN **SP. NOV.**

Etymology: Refers to the obvious conflict between morphology and molecular data. The recommended Chinese name is “矛盾越田螺”.

Material examined

Holotype: ZBMFD.20220900GXBSSL1, living collected specimen, caught by a sand dredger boat from the sand bottom in the middle of You River (minimum depth around 8 m), Silin Town, Tiandong County, Baise City, Guangxi Province, China, 9.2022. Paratypes: 7 living collected specimens, same collecting data as the holotype, 3 kept in ZBMFD (ZBMFD.20220900GXBSSL2), 1 kept in ZMB (ZMB.122765), 4 kept in ZLJ (22BSSL) (Fig.35).

Description

Shell (Fig.34A-E) medium, thick, solid, broadly conical or subglobose, olive to greenish brown, with five whorls at adulthood, teleoconch whorls without shoulder, normally with three strong smooth keels, sometimes with one weaker keel located between two smooth keels, base of shell smooth or with two to three weak keels, keels usually darker than the other part of shell surface in colour; aperture ovate, large, nearly half of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thick nacreous layers, white, outward folding, completely covering umbilicus.

Operculum (Fig.34F-G) corneous, ovate, relatively thin, much smaller than shell aperture, yellow to orange; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.36) (n=2) central tooth with one broad central denticle and four small sharp cusps on either side; lateral tooth with one broad central denticle and four small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and three small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 29 to 30 small sharp cusps.

Remarks

Bakyietaia paradoxa can be easily distinguished morphologically from all its congeners based on its subglobose thick shell with strong keels, a wide aperture and a very small operculum. This species is common at the type locality and not sympatric with any other *Bakyietaia* species. Besides, it is only discovered in the deep water region of a big river, which is distinct from the habitats of all other *Bakyietaia* species. However, the COI tree revealed that this species clusters undistinguishably in one clade with *Bakyietaia boettgeri*, which has a completely different conical shell with a relatively small aperture and big operculum. In

addition, *B. paradoxa* has more cusps on the outer marginal teeth than *B. boettgeri* (29-30 vs 23-24).

Habitat and distribution

Only known from the sand substrate of deep water in You River, Guangxi Province, China.

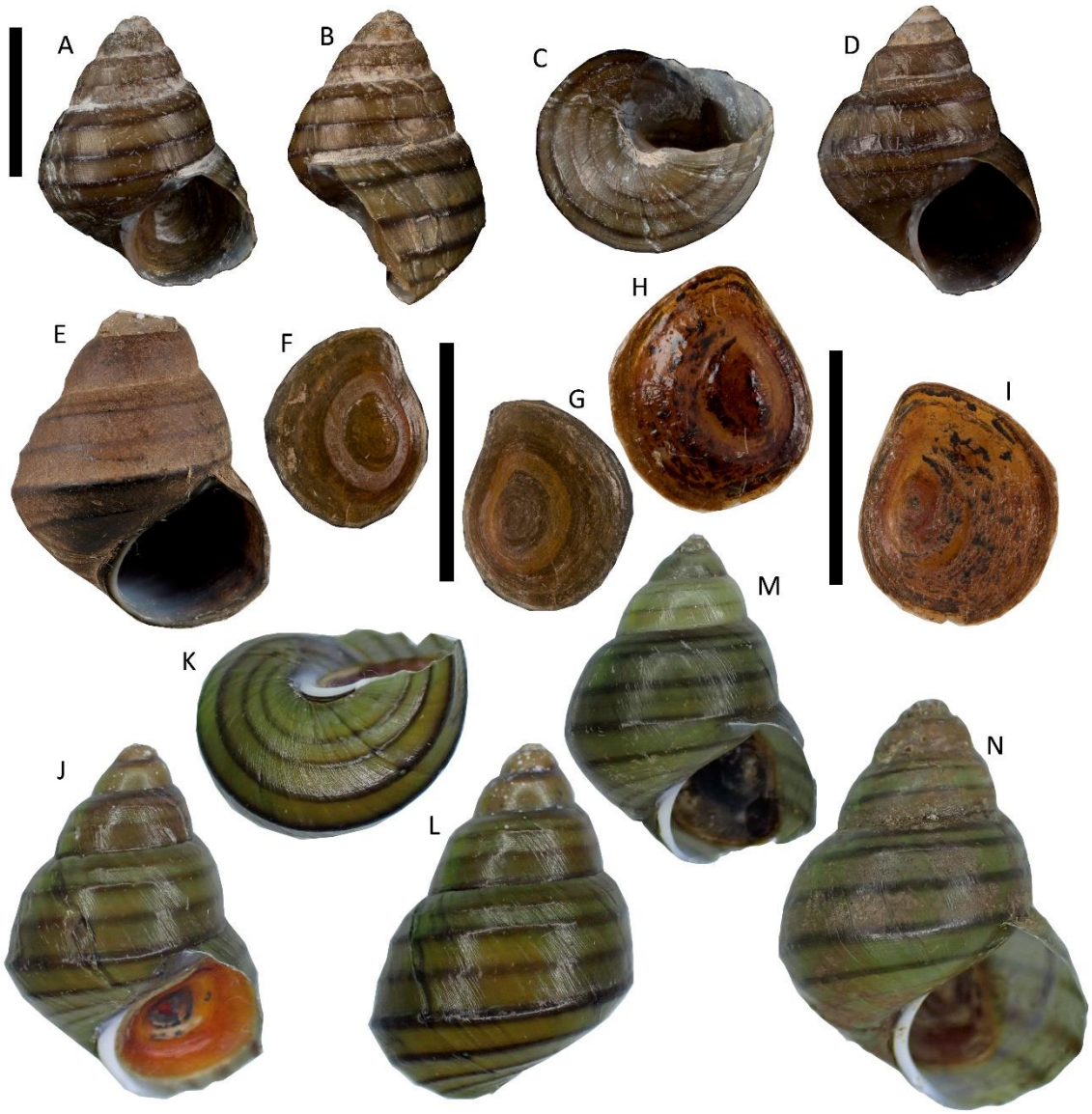


Figure 37. Shells and operculum of *Bakyietaia avisvenatoris*: A-C, holotype, ZBMFD.20200618GDGZSC1, Paitan Town, Guangdong, China; D, paratype, ZMB.122757, Paitan Town, Guangdong, China; E, paratype, ZMB.122698, Shuikou, Guangxi, China; F-G, operculum of ZMB.122757; H-I, operculum of ZMB.122698; J-L, paratype, ZBMFD.20200618GDGZSC2-1, Shuikou, Guangxi, China; M-N, paratypes, 20200618GDGZSC2-2/3, Baishilin, Guangxi, China; scale bars=1 cm, top left bar applies to all shells, separate bar for opercula F-I.

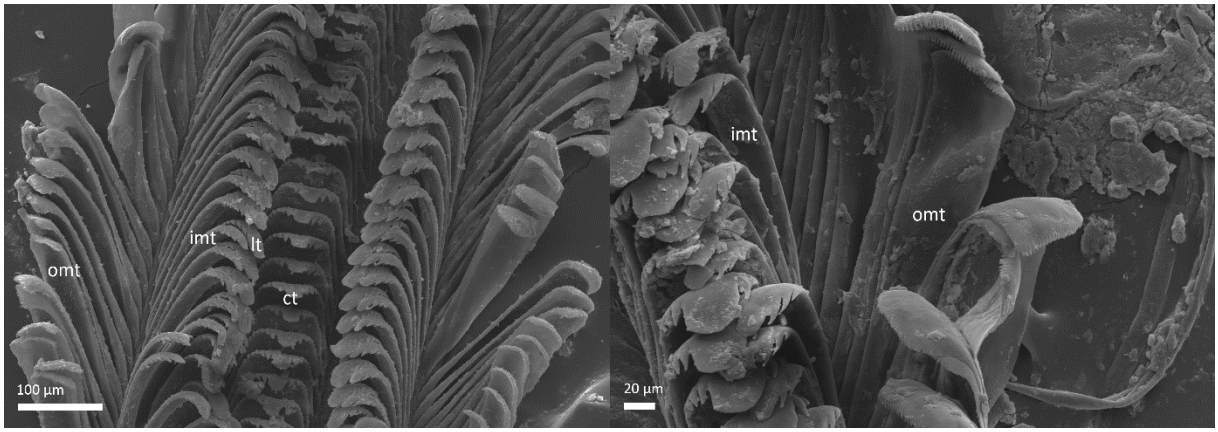


Figure 39. SEM photo of the radula of *Bakyietaia avisvenatoris*, ZMB. 122757 (left), ZMB.122698 (right). Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA AVISVENATORIS YEN & ZHANG **SP. NOV.**

Etymology: In memory of one of the ancestors of the type material collector, who was a bird hunter and moved to the type locality due to the abundance of birds there. The recommended Chinese name is "猎人越田螺".

Material examined

Holotype: ZBMFD.20200618GDGZSC1, living collected specimen, a pond in Shi Village, Paitan Town, Zengcheng District, Guangzhou City, Guangdong Province, China, 18.6.2020, leg. Da-Ming Shi. Paratypes: 4 living collected specimen, same collecting data as the holotype, 2 kept in ZBMFD (ZBMFD.20200618GDGZSC2), 1 kept in ZMB (ZMB.122757), 1 kept in ZLJ (20PT); 5 living collected specimen, 0.4 m deep on mud substrate or rock, Wangyao/Baishilin/Shuikou, Wangsha Village, Luchuan County, Yulin City, Guangxi Province,China, 15.11.2019, leg. Bei-Hua Liang, 3 kept in ZBMFD (ZBMFD.20200618GDGZSC2), 1 kept in ZMB (ZMB.122698), 1 kept in ZLJ (20GZPT) (Fig.38).

Description

Shell (Fig.37A-E, J-N) relatively small for the genus, thin but solid, conical, olive to olive brown, with five whorls at adulthood, apex acute; teleoconch whorls not inflated, sometimes with shoulder, spiral forming a triangle outline, with three smooth main keels, with one to two weaker smooth keel located between suture and the first keel, sometimes one additional weak keel located between first and second/second and third keels, base of shell with one to three weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thin nacreous layers, white, inner lip outward folding, completely or partly covering umbilicus.

Operculum (Fig.37F-I) corneous, ovate, relatively thin, slightly smaller than shell aperture, yellow to red; exterior surface smooth; interior surface with relatively small inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.39) (n=2) central tooth with one broad central denticle and four to five small sharp cusps on either side; lateral tooth with one broad central denticle and four to five small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and three to four small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 24 to 27 small sharp cusps.

Remarks

Bakyietaia liangzhuorum can be distinguished from its congeners based on its relatively small conical shell with the triangle outline and outward folding thin inner lip.

Habitat and distribution

Mud substrate or rock in ponds and creeks in eastern Guangxi Province and central Guangdong Province, China.

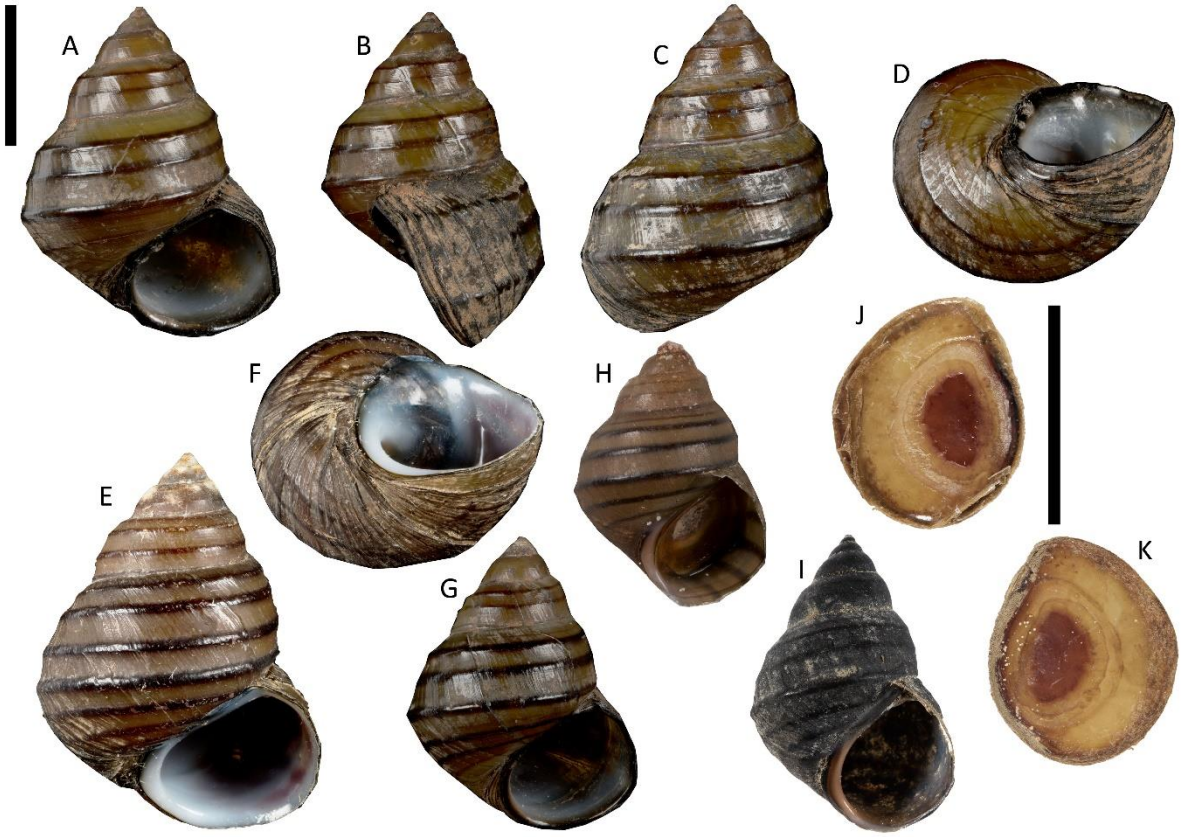


Figure 40. Shells and operculum of *Bakyietaia liangzhuorum*: A-D, holotype, ZBMFD.20191220GXLBJC1, Laibin, Guangxi, China; E-F, paratype, ZBMFD.20220924GDQYST1, Shitan Town, Guangdong, China; G, paratype, ZMB.122691,

Laibin, Guangxi, China; H, paratype, ZMB.122761, Qinzhou, Guangxi, China; I, paratype, ZMB.122719, Chenghuang Town, Guangxi, China; J-K, operculum of ZBMFD.20191220GXLBJC1; scale bars=1 cm, top left bar applies to all shells, separate bar for operculum J-K.

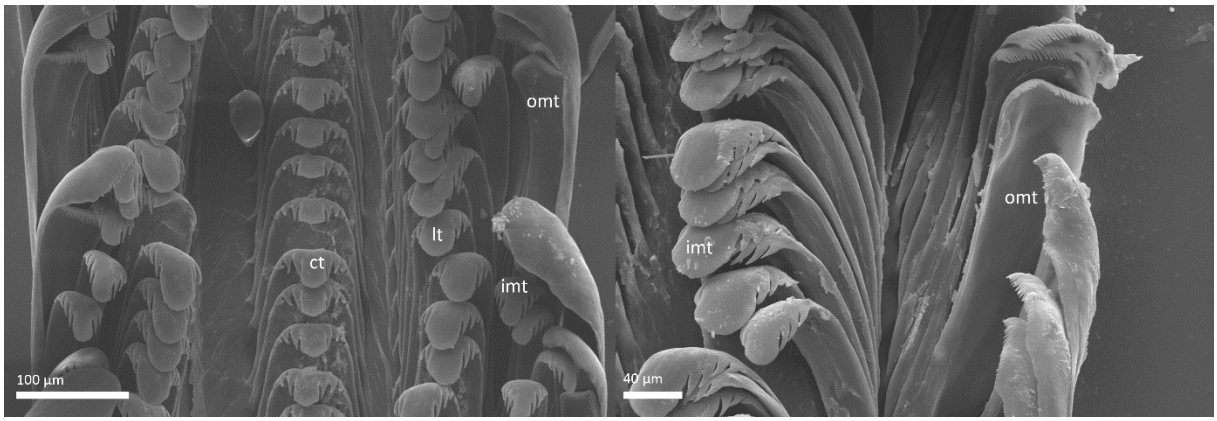


Figure 42. SEM photo of the radula of *Bakyietaia liangzhuorum*, ZMB.122691 (left), ZMB.122719 (right). Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA LIANGZHUORUM YEN & ZHANG **SP. NOV.**

Etymology: "Liang Zhu" is the abbreviated title of one of China's four great folktales, "Liang Shanbo and Zhu Yingtai". They loved each other, but Zhu was forcibly betrothed to someone else. Liang, grief-stricken, became ill and died. When Zhu's wedding procession passed Liang's tomb, a strong wind suddenly blew up, forcing them to stop. In front of the tomb, Zhu told Liang how much she loved him. The tomb burst open and Zhu immediately jumped into it. Afterwards a pair of colourful butterflies appeared from the tomb: their spirits emerged in the form of butterflies and flew away together, never to be separated again.

Inspiration for the name liangzhuorum for this species is drawn from seven ancient tombs of the Han Dynasty (202 BC- 220 AD) in Basi, which are located near type locality. The recommended Chinese name is "彩蝶越田螺".

Material examined

Holotype: ZBMFD.20191220GXLBJC1, living collected specimen, 0.5 m deep in crack of rocks, a river in Jin Village, Tongwan Town, Wuxian County, Laibin City, Guangxi Province, China, 20.12.2019, leg. Han-Zhi Tan. Paratypes: 9 living collected specimens, same collecting data as the holotype, 4 kept in ZBMFD (ZBMFD.20191220GXLBJC2), 1 kept in ZMB (ZMB.122691), 4 kept in ZLJ (19LB); 4 living collected specimens, 0.1 m deep on mud and sand substrate, a river near Shirentou Mountain, Chenghuang Town, Yulin City, Guangxi Province, China, 13.3.2022, leg. Le-Jia Zhang, 2 kept in ZBMFD (ZBMFD.20191220GXLBJC2), 1 kept in ZMB (ZMB.122719), 1 kept in ZLJ; 4 living

collected specimens, 0.6 m deep on rock with sand and mud substrate, Bin River near Shitan Town, Qingxin District, Qingyuan City, Guangdong Province, China, 24.9.2022, leg. Mr.Chen, 2 kept in ZBMFD (ZBMFD.20220924GDQYST1), 1 kept in ZMB (ZMB. 122762), 1 kept in ZLJ (22QYST); 10 living collected specimens, 0.2 m deep on mud substrate, Hengtong, Liuken Town, Pubei County, Qinzhou City, Guangxi Province, China, 25.9.2020, leg. Mr. Huang, 4 kept in ZBMFD (ZBMFD.20200925GXQZHT1), 2 kept in ZMB (ZMB.122761), 4 kept in ZLJ (20QZ) (Fig.41).

Description

Shell (Fig.39A-I) medium, thin to thick, solid, conical, olive to greenish brown, with six whorls at adulthood, teleoconch whorls not inflated, spiral forming a triangle outline, with three smooth strong main keels, with one to two weak smooth keel located between suture and the first keel, sometimes one additional weak keel located between first and second/second and third keels, base of shell with one to four weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white to pale purple, margin of outer and inner lip dyed black or white, outer lip slightly thickened, inner lip covered with thick nacreous layers, white or pale orange, inner lips of the last growing stages outward folding and tightly overlapping, forming a relatively narrow crescent callus-like structure, this structure completely covering umbilicus.

Operculum (Fig.39J-K) corneous, ovate, relatively thick, slightly smaller than shell aperture, yellow to orange, sometimes with red nuclear region; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.42) (n=5) central tooth with one broad central denticle and three to four small sharp cusps on either side; lateral tooth with one broad central denticle and three to four small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and three to four small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 20 to 25 small sharp cusps.

Remarks

Bakyietaia liangzhuorum can be distinguished from its congeners based on its medium-sized conical shell with a triangle outline, strong main keels and thickened outer lip. This species occurs sympatrically with *B. chenghuang* in Chenghuang Town. However, *B. liangzhuorum* prefers a sandy/muddy substrate, while *B. chenghuang* dwells on big rocks.

Habitat and distribution

Mostly Sand or mud substrate (occasionally on rock) of rivers in Eastern Guangxi Province and Central Guangdong Province, China.

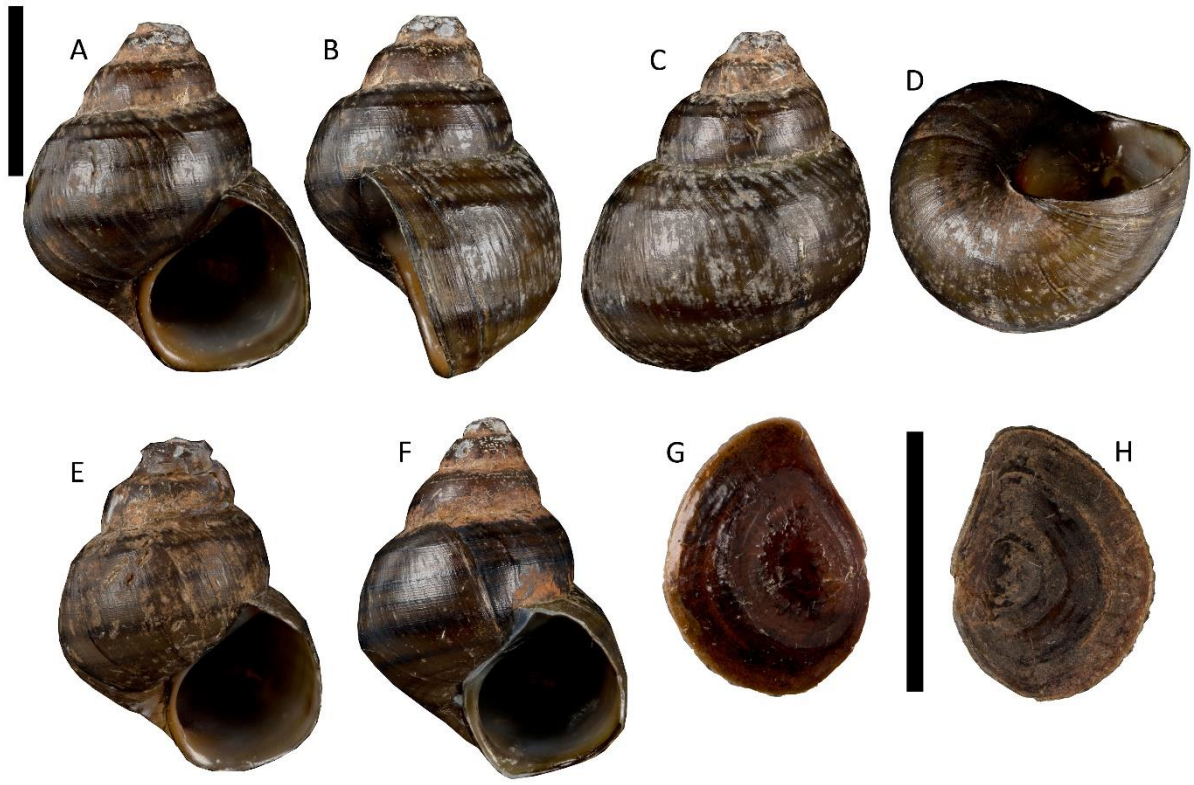


Figure 43. Shells and operculum of *Bakyietaia luikongi*: A-D, holotype, ZBMFD.20200206GDZJLR1, Leizhou, Guangdong, China; E-F, paratype, ZMB.122764, Leizhou, Guangdong, China; G-H, operculum of ZBMFD.20200206GDZJLR1; scale bars=1 cm, top left bar applies to all shells, separate bar for operculum G-H.

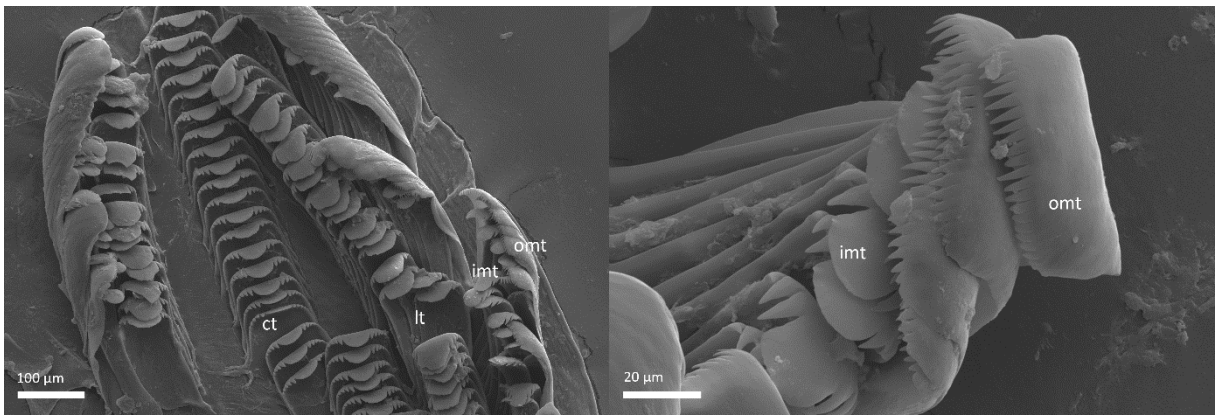


Figure 45. SEM photo of the radula of *Bakyietaia luikongi*, ZMB. 122764. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA LUIKONGI YEN & ZHANG **SP. NOV.**

Etymology: Named after “Lui Kong”, the god of thunder in local Min Nan Chinese dialect, popular in the folk believe of Leizhou County, the type locality of this species. The recommended Chinese name is “雷公越田螺”.

Material examined

Holotype: ZBMFD.20200206GDZJLR1, living collected specimen, 0.1-0.5 m deep mud and rock substrate in ponds, near Nanguang Farm Second Team, Liren Village, Leizhou County, Zhanjiang City, Guangdong Province, China, 6.2.2020, leg. Yu-Xuan Zhou. Paratypes: 9 living collected specimens, same collecting data as the holotype, 4 kept in ZBMFD (ZBMFD.20200206GDZJLR2), 2 kept in ZMB (ZMB.122764), 3 kept in ZLJ (20LZLR) (Fig.44).

Description

Shell (Fig.43A-F) relatively small for the genus, thin but solid, broad conical to conical, dark olive brown, with five whorls at adulthood, apex acute; teleoconch whorls sometimes with shoulder, with three weak but obvious smooth main keels, with one to two weaker smooth keel located between suture and the first keel, always with one weak keel located between first and second/second and third keels, base of shell with three to four weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thin nacreous layers, white to orange, inner lip outward folding, completely covering umbilicus.

Operculum (Fig.43G-H) corneous, ovate, thin, slightly smaller than shell aperture, dark orange to dark red; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.45) (n=2) central tooth with one broad central denticle and four small sharp cusps on either side; lateral tooth with one broad central denticle and four small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and three to four small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 19 to 20 small sharp cusps.

Remarks

Bakyietaia luikongi is the only “weak main keels” *Bakyietaia* species distributed outside Hainan Island and the sister species of the clade including all “weak main keels” species from Hainan. It can be easily distinguished from all congeners based on the small thin conical shell with three very weak main keels and without crescent callus-like structure. It has the smallest shell within the “weak main keels” group. The geographical separation affected by the Hainan Strait between Hainan Island and Leizhou Peninsula probably played a role in the origin of this species.

Habitat and distribution

Only known from the mud and rock substrate in ponds of Leizhou Peninsula, Guangdong Province, China.

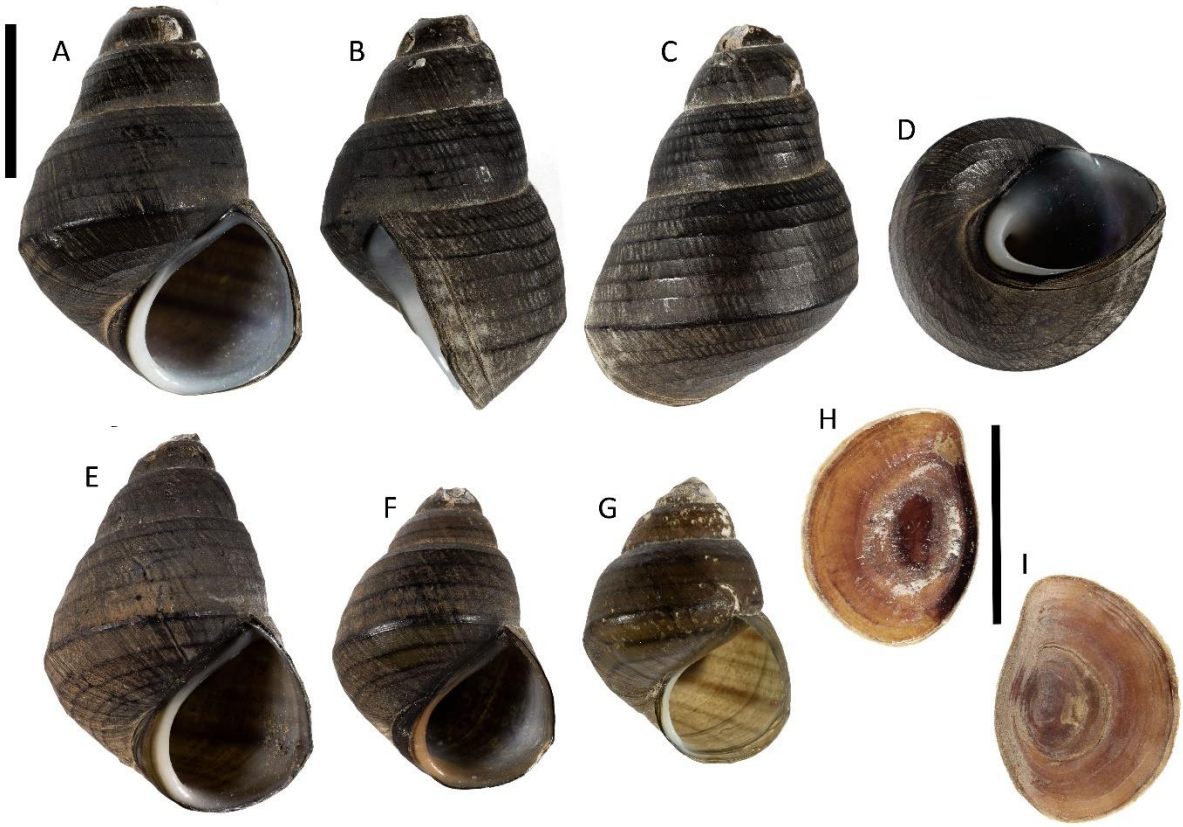


Figure 46. Shells and operculum of *Bakyietaia fontinalis*: A-D, holotype, ZBMFD.20220306HNDAJWT1, Jiuwentang, Hainan, China; E-F, paratype, ZMB.122764, Jiuwentang, Hainan, China; G, paratype, ZLJ.22WQH, Wanquan River, Hainan, China; H-I, operculum of ZBMFD.20220306HNDAJWT1; scale bars=1 cm, top left bar applies to all shells, separate bar for operculum H-I.

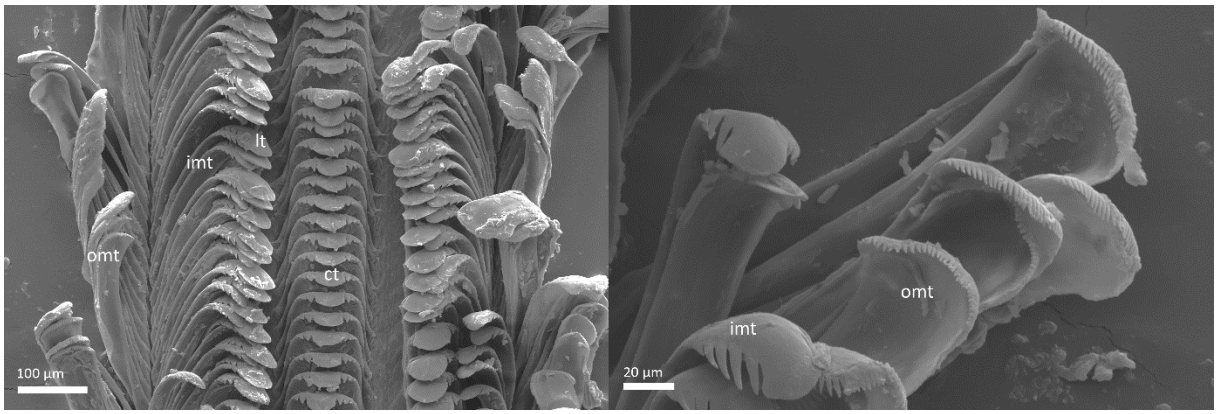


Figure 48. SEM photo of the radula of *Bakyietaia fontinalis*, ZMB. 122733. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA FONTINALIS ZHANG & YEN **SP. NOV.**

Etymology: Refers to cold spring habitat of the type locality. The recommended Chinese name is “冷泉越田螺”.

Material examined

Holotype: ZBMFD.20220306HNDAJWT1, living collected specimen, 0.5 m deep on mud and sand substrate, Jiuwentang Cold Spring, Jiuwentang Town, Dingan County, Hainan Province, China, 6.3.2022/3/6, leg. Le-Jia Zhang. Paratypes: 4 living collected specimens, same collecting data as the holotype, 1 kept in ZBMFD (ZBMFD.20220306HNDAJWT2), 2 kept in ZMB (ZMB.122733), 1 kept in ZLJ (22DAJWT); 2 living collected specimens, river near Leiming Town, Dingan County, Hainan Province, China, year 2018, kept in ZLJ; 1 living collected specimens, 0.1 m deep on rock, Wanquan River, Shibi Town, Qionghai City, Hainan Province, China, 7.3.2022, leg. Le-Jia Zhang, kept in ZLJ (22WQH) (Fig.47).

Description

Shell (Fig.46A-G) medium, thin to thick, solid, conical, olive to dark olive brown, with five whorls at adulthood, apex acute; teleoconch whorls not inflated, spiral always forming a triangle outline, with three weak but obvious smooth main keels, with one to two weaker smooth keel located between suture and the first keel, always with one additional weak keel located between first and second/second and third keels, base of shell with two to four weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thick nacreous layers, white or orange, inner lips of the last growing stages outward folding and tightly overlapping, sometimes forming a crescent callus-like structure, this structure or inner lip completely covering umbilicus.

Operculum (Fig.46H-I) corneous, ovate, thin, slightly smaller than shell aperture, orange to dark red; exterior surface smooth; interior surface with relatively small inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.48) (n=2) central tooth with one broad central denticle and four to five small sharp cusps on either side; lateral tooth with one broad central denticle and four small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and four to five small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 26 to 29 small sharp cusps.

Remarks

Bakyietaia fontinalis is one of the “weak main keels” *Bakyietaia* species. This species can be easily distinguished from all congeners, especially the morphologically similar species *B. mutica*, based on its medium-sized conical shell with non-inflated whorls, three weak main

keels and three weak additional keels and more cusps on the outer marginal teeth (26-29 vs 19-24).

Habitat and distribution

Mud and rock substrate in springs and rivers of Central-eastern Hainan Province, China.

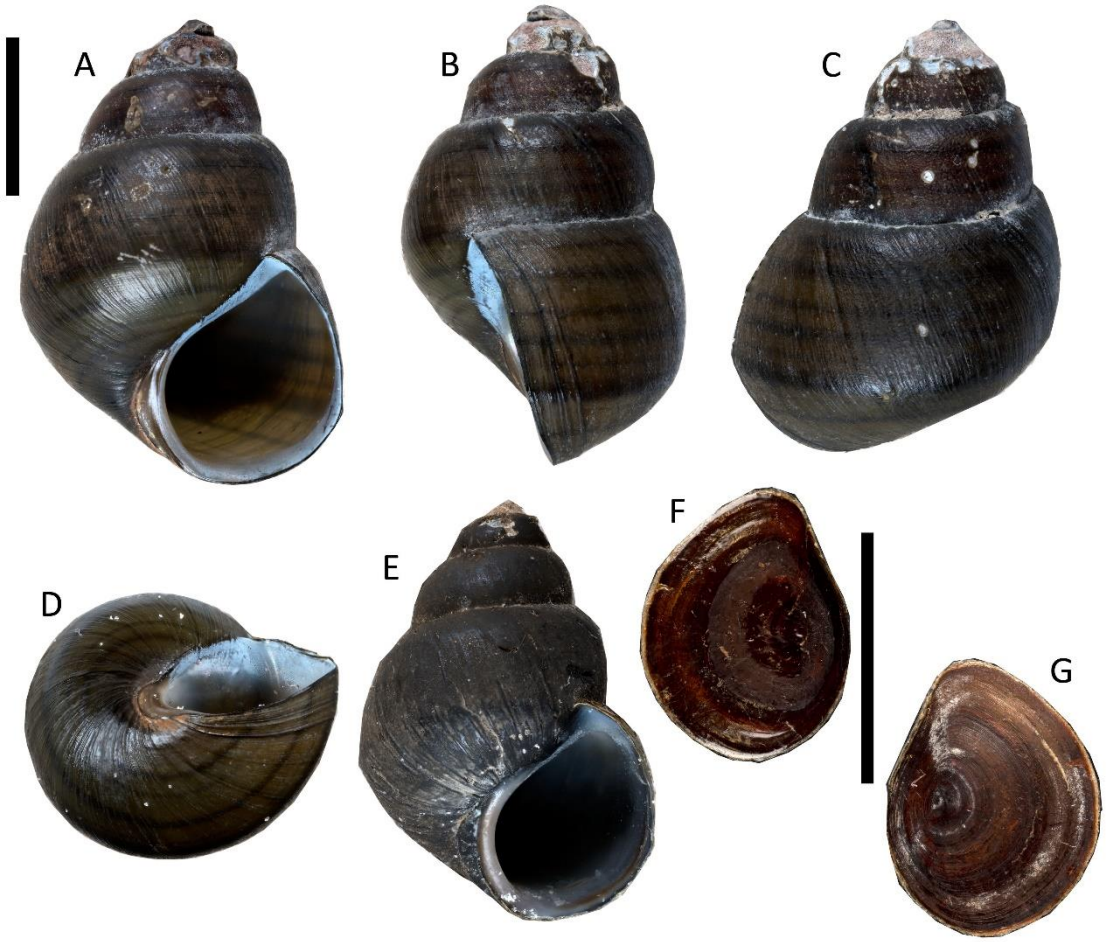


Figure 49. Shells and operculum of *Bakyietaia naiadica*: A-D, holotype, ZBMFD.20220305HNHKLP1, Longpo Temple, Hainan, China; E, paratype, ZBMFD.20220305HNHKXC1, Xue Village, Hainan, China; F-G, operculum of ZBMFD.20220305HNHKLP1; scale bars=1 cm, top left bar applies to all shells, separate bar for operculum F-G.

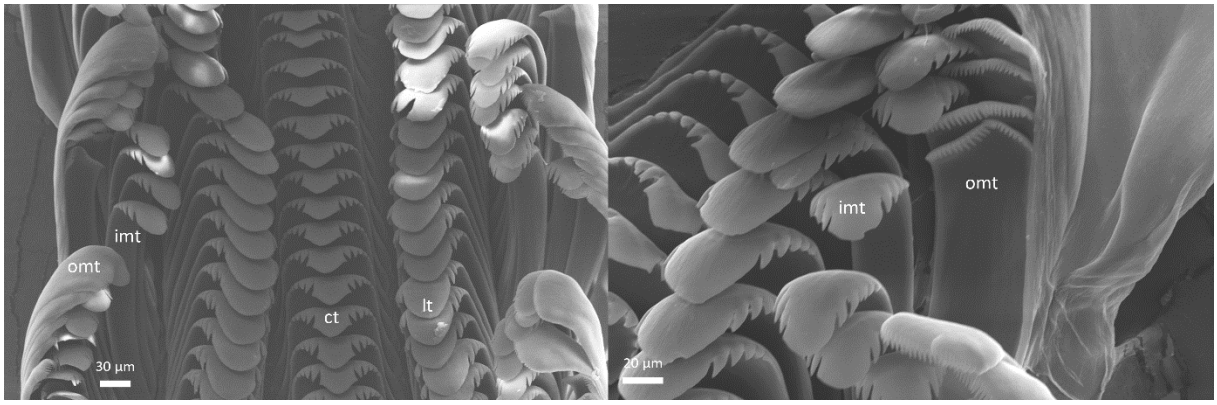


Figure 51. SEM photo of the radula of *Bakyietaia naiadica*, ZMB. 122743. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA NAIADICA ZHANG & YEN **SP. NOV.**

Etymology: Named after Naiad, the nymph of springs in Greek mythology. The recommended Chinese name is “龙婆越田螺”.

Material examined

Holotype: ZBMFD.20220305HNHKLP1, living collected specimen, 0.1 m deep on rock, spring near Longpo Temple, Haikou City, Hainan Province, China, 5.3.2022, leg. Le-Jia Zhang. Paratypes: 1 living collected specimen, same collecting data as the holotype, kept in ZMB (ZMB.122743); 1 living collected specimen, 0.1 m deep on rock substrate, wetland in Xue Village, Haikou City, Hainan Province, China, 3.5.2022, leg. Le-Jia Zhang, kept in ZBMFD (ZBMFD.20220305HNHKXC1) (Fig.50).

Description

Shell (Fig.49A-E) medium, thin but solid, conical, olive to dark olive brown, with five whorls at adulthood; teleoconch whorls inflated, with shoulder, with three very weak smooth main keels, with one to two even weaker smooth keel located between suture and the first keel, sometimes with one additional weak keel located between first and second/second and third keels, base of shell with two to four weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thick nacreous layers, white or pale orange, inner lips of the last growing stages outward folding and tightly overlapping, sometimes forming a crescent callus-like structure, this structure completely covering umbilicus.

Operculum (Fig.49F-G) corneous, ovate, thin, slightly smaller than shell aperture, dark red; exterior surface smooth; interior surface with relatively small inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.51) (n=1) central tooth with one broad central denticle and four to five small sharp cusps on either side; lateral tooth with one broad central denticle and four to five small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and five to six small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 19 to 22 small sharp cusps.

Remarks

Bakyietaia naiadica can be easily distinguished from all congener species, especially morphologically similar species *B. mutica*, based on its more cylindrical outline of shell (vs triangle outline of shell in *B. mutica*) and the weaker main keels.

Habitat and distribution

Rock substrate in springs and wetlands of Northern Hainan Province, China.

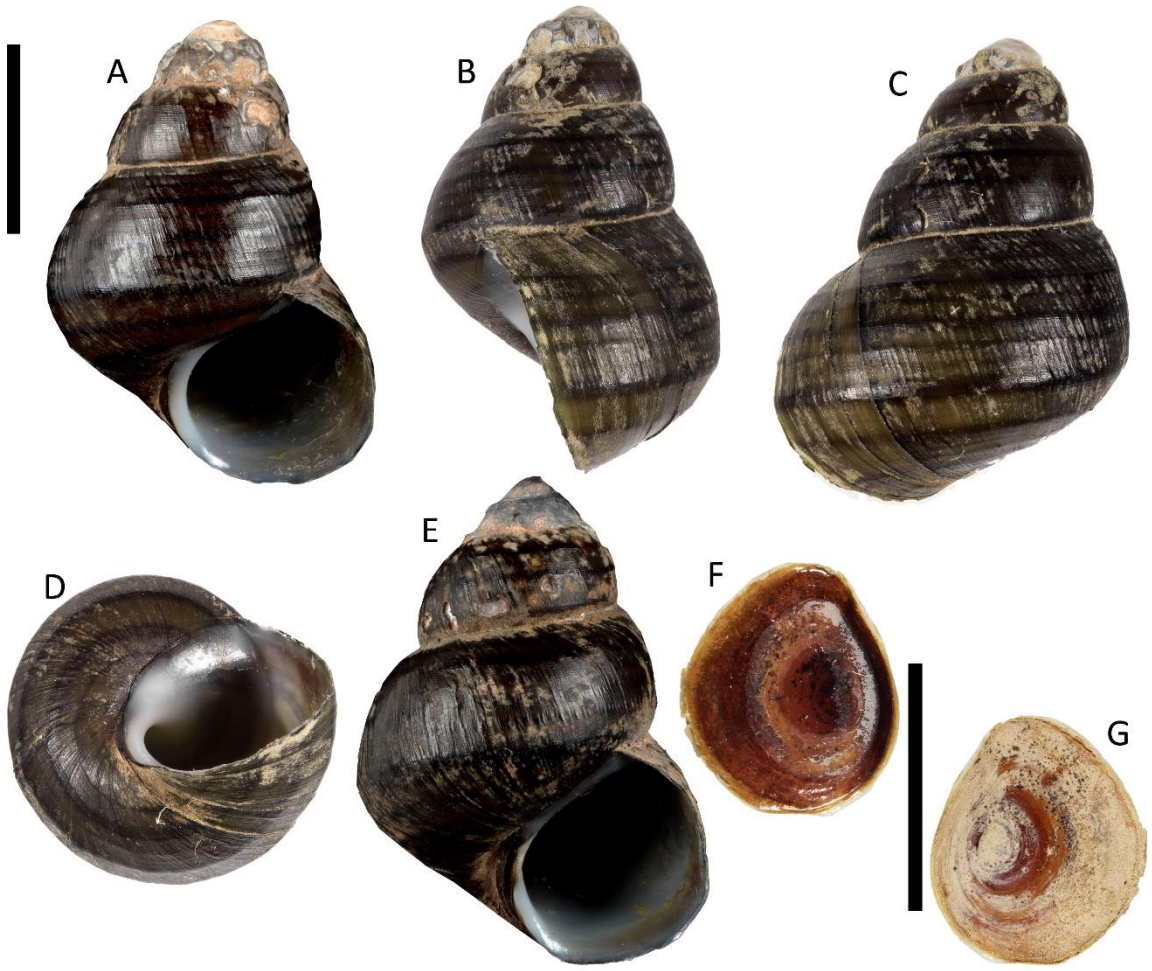


Figure 52. Shells and operculum of *Bakyietaia luuemxlong*: A-D, holotype, ZBMFD.20220307HNQHMT1, Mutang, Hainan, China; E, paratype, ZMB.122739, Mutang, Hainan, China; F-G, operculum of ZBMFD.20220307HNQHMT1; scale bars=1 cm, top left bar applies to all shells, separate bar for operculum F-G.

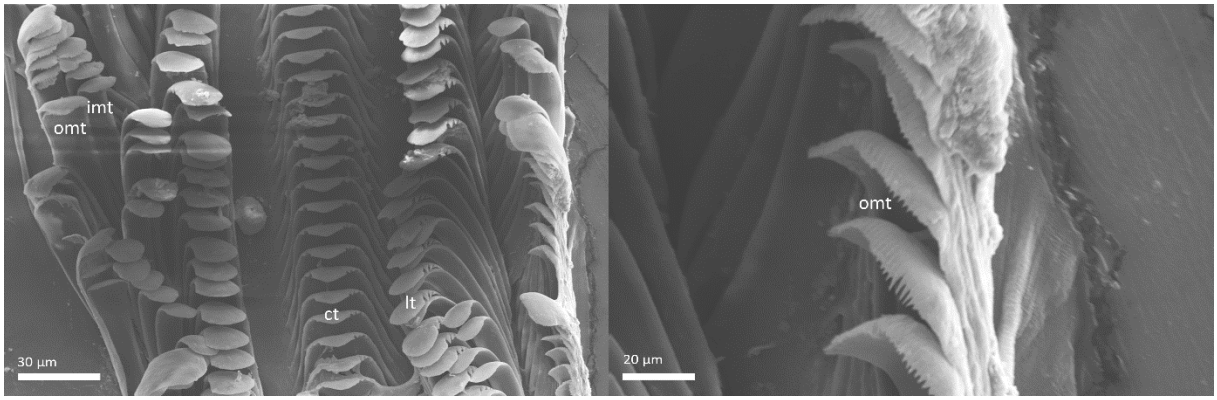


Figure 54. SEM photo of the radula of *Bakyietaia luuemxlong*, ZMB. 122739. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA LUUEMXLONG ZHANG & YEN **SP. NOV.**

Etymology: "luuemx long", which means "forget to grow up" in Hlai language, is a race of dwarves living in the forest according to the mythology of Hlai people from Hainan Island. Formed as a noun in apposition. The recommended Chinses name is "阑咙越田螺".

Material examined

Holotype: ZBMFD.20220307HNQHMT1, living collected specimen, 0.1 m deep on mud substrate, creeks near farmland of Mutang Village, Huishan Town, Qionghai City, Hainan Province, China, 2022/3/7, leg. Le-Jia Zhang. Paratypes: 3 living collected specimen, same collecting data as the holotype, 2 kept in ZBMFD (ZBMFD.20220307HNQHMT2), 1 kept in ZMB (ZMB.122739) (Fig.53).

Description

Shell (Fig.52A-E) small to medium, thin but solid, conical, dark olive brown, with five whorls at adulthood; teleoconch whorls inflated, spiral sometimes forming a triangle outline, with three weak but obvious main keels, with one to two even weaker smooth keel located between suture and the first keel, always with one additional weak keel located between first and second/second and third keels, base of shell with two to four weak keels, all keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thick nacreous layers, white, inner lip outward folding, covering umbilicus.

Operculum (Fig.52F-G) corneous, ovate, thin, slightly smaller than shell aperture, dark red; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins or relatively smooth glossy surface.

Radular (Fig.54) (n=1) central tooth with one broad central denticle and four small sharp cusps on either side; lateral tooth with one broad central denticle and three small sharp cusps

on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and unknown number of small sharp cusps on either side (all heavily worn); outer marginal tooth wide, almost same as lateral tooth in width, with around 20 small sharp cusps.

Remarks

Bakyietaia luuemxlong is one of the "weak main keels" *Bakyietaia* species. This species can be easily distinguished from all congeners based on the small to medium-sized conical shell with inflated whorls and three weak main keels and three weak additional keels.

Habitat and distribution

Only known from the type locality with mud substrate in creeks of central-eastern Hainan Province, China.

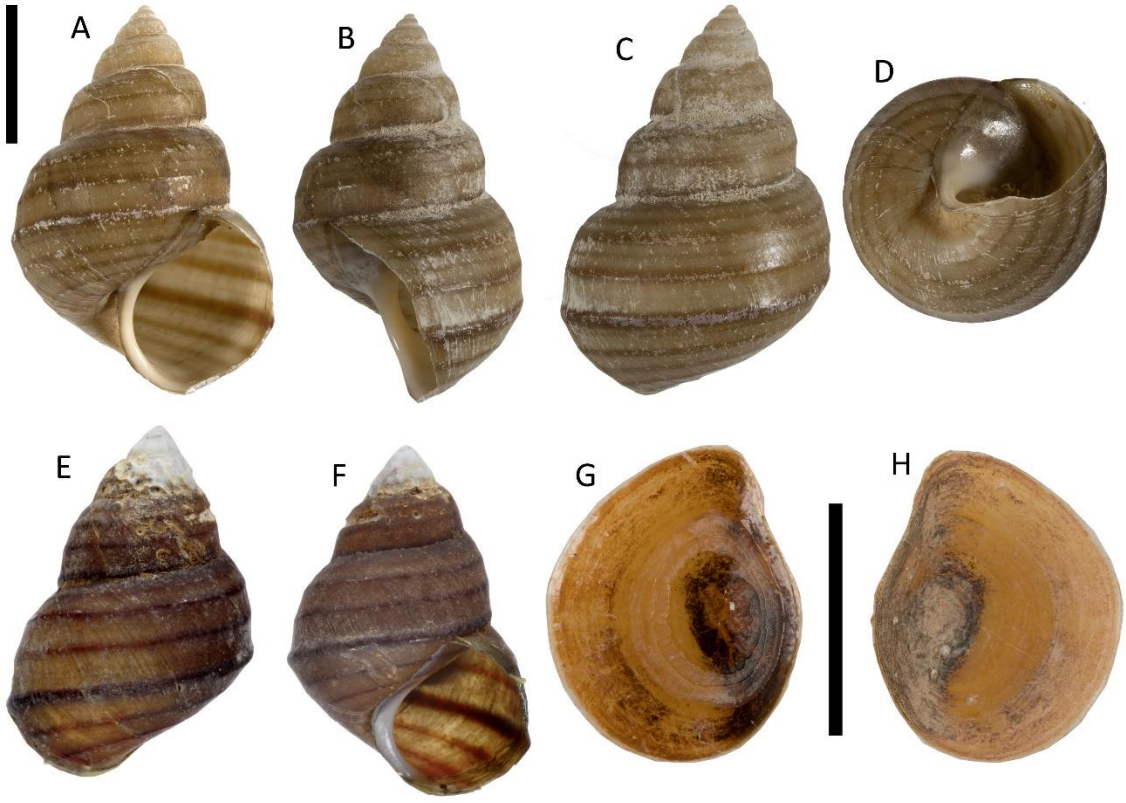


Figure 10. Shells and operculum of *Bakyietaia indrapura*: A-D, holotype, ZMB.114402.1, Nghe An, Vietnam; E-F, paratype, ZMB.114403, Phuoc Son, Vietnam; K-L, operculum of 114402; scale bar=1 cm, All shells & operculum J-K separately with the same scale bar.

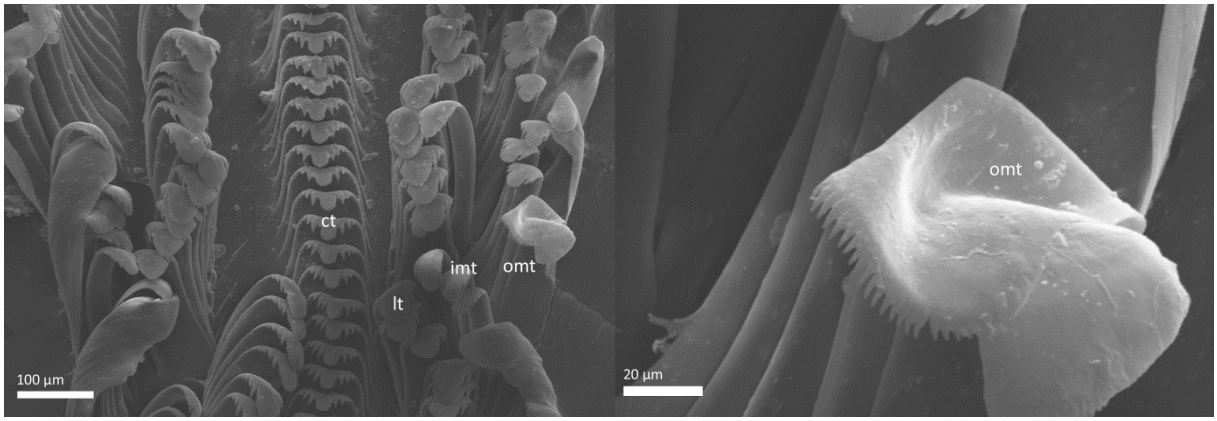


Figure 12. SEM photo of the radula of *Bakyietaia indrapura*, ZMB.114402.1. Abbreviations: ct–central teeth; lt–lateral teeth; imt–inner marginal teeth; omt–outer marginal teeth.

BAKYIETAIA INDRAPURA ZHANG & YEN **SP. NOV.**

Etymology: Named after the capital of the ancient kingdom Champa, Indrapura, where is close to the type locality. Formed as a noun in apposition. The recommended Chinses name is "占婆越田螺".

Material examined

Holotype: ZMB.114402.1, 1 living-collected specimens, montain creek, Truong Son Mts., Tam Quang, Nghe An, Vietnam, 21-31.10.2006, leg. Frank Köhler. Partypes: ZMB.114402.2-5, 4 living collected specimen, same collecting data as the holotype; ZMB.114403, 3 living-collected specimens, Cai River drainage, Kham Duc, Phuoc Son, Vietnam, 21-31.10.2006, leg. Frank Köhler; ZMB.114406, 3 living-collected specimens, montain creek, Truong Son Mts., Tam Quang, Tân Duong District, Vietnam, 21-31.10.2006, leg. Frank Köhler.

Description

Shell (Fig.10A-J) medium, thin but solid, conical, olive to greenish brown, with five to six whorls at adulthood, apex acute; teleoconch whorls, especially body whorl, slightly inflated, sometimes forming shoulder, with three smooth main keels, with one to two weaker smooth keel located between suture and the first keel, sometimes one additional weak keel located between first and second/second and third keels, base of shell with three to five weak keels, keels usually darker than the other part of shell surface in colour; aperture ovate, less than half of shell in height, inner shell surface bluish white, outer lip thin and simple, inner lip covered with thick nacreous layers, white or orange, inner lips of the last growing stages outward folding and tightly overlapping, forming a very narrow crescent callus-like structure or without this structure, inner lip or this structure usually covering umbilicus, umbilicus sometimes narrowly open, bordered by a keel.

Operculum (Fig.10K-L) corneous, ovate, thin, slightly smaller than shell aperture, yellow to orange; exterior surface smooth; interior surface with relatively large inner opercular region, nuclear region small, with shiny grains or veins.

Radular (Fig.12) (n=4) central tooth with one broad central denticle and five small sharp cusps on either side; lateral tooth with one broad central denticle and five small sharp cusps on either side; inner marginal tooth narrow, about half of lateral tooth in width, with one broad central denticle and five to six small sharp cusps on either side; outer marginal tooth wide, almost same as lateral tooth in width, with 21 to 26 small sharp cusps.

Remarks

The newly collected specimens from Nghe An of Vietnam have a shell that is highly similar to that of the lectotype. *Bakyietaia polyzonata* can be distinguished from most congeners by its medium-sized thin conical shell with inflated whorls. The type locality of this species was not clear from the original description, and it was considered as China (Yen, 1939). However, this species has never been found in China based on historic and recent materials. The distribution of *Bakyietaia polyzonata* in central Vietnam and Laos is far from the distributions of other congeners. It is probably the southernmost native species of this genus.

Habitat and distribution

Rivers of Central Vietnam.

DISCUSSION

The present study is the first part of the revision of the "wastebasket taxon" *Angulyagra*. Although no material of the type species *Angulyagra oxytropis* has been available for the molecular phylogenetic part of this study, the data from the investigation of the shell, operculum, radula and anatomy strongly supports the assignment of the so-called "*Angulyagra"* species from South China and Mainland Southeast Asia to a new genus, *Bakyietaia*. In addition, the distribution of *Angulyagra oxytropis* in Manipur, India is far from the known distribution of the new genus. A future molecular study including fresh material of *Angulyagra oxytropis* from Manipur will help to further understand the relationship between *Angulyagra* and *Bakyietaia*.

The integrative approach combining morphology, anatomy and COI sequence data has confirmed the validity of the new genus *Bakyietaia*. All morphologically similar genera from China and Southeast Asia, such as *Sinotaia*, *Filopaludina*, *Anulotaia* and *Taia*, are shown to be distinct genera based on the COI phylogeny (Fig. 2), not least by the large p-distances between *Bakyietaia* and other genera (8.45%-15.65%). Our COI tree is broadly consistent with the genus-level topology of the subfamily Bellamyinae Rohrbach, 1937 based on

multiple genes in Stelbrink *et al*. (2020). *Bakyietaia* falls within the clade B of Bellamyinae proposed by Stelbrink *et al*. (2020), which is widespread in Africa, Southeast Asia and Australia.

A lot of former studies have shown that the species-level phylogeny solely based on single or several mitochondrial markers is problematic in Viviparidae (Zhang *et al*., 2015; Hirano *et al*., 2019b; Ye *et al*., 2021). However, in the present study, the monophyly of most *Bakyietaia* species is highly supported in the COI tree. We found a high degree of congruence between morphology-based species delimitations and species level units inferred from the COI phylogeny. Within the 16 recognised morphospecies, only one species, *Bakyietaia paradoxa*, cannot be differentiated from *Bakyietaia boettgeri* by the COI marker. *Bakyietaia paradoxa* is considered as a valid species with recent divergence because of its distinct shell, operculum, radula and its unique habitat in deep water, which is not found in any other *Bakyietaia* species. A more detailed ecological and genomic study would be desirable to uncover the patterns of adaption and speciation in this species. At this point, the sharing of mtDNA haplotypes is compatible with either introgressive hybridization or incomplete lineage sorting (Hirano *et al*., 2019b; Glasheen *et al*., 2020).

The radula is considered valuable in the classification of viviparid species (Zhang *et al*., 2015; Zhang *et al*., 2023). In other freshwater snails, radula diversification has been considered to be driven by trophic specialization, which promotes adaptive radiations of different species (von Rintelen *et al*. 2004; Hilgers *et al*., 2022). *Bakyietaia* displays a high interspecific radula diversity, especially regarding the number of cusps on the outer marginal teeth. Several species with similar shells can be differentiated based on radula characters, such as *B. subcostata* and *B. chenghuang*, *B. guangdungensis* and *B. wilhelmi*, *Bakyietaia mutica* and *B. fontinalis*. In one instance, two species occur sympatrically at the same site in a creek near Chenghuang Town, *B. chenghuang* and *B. liangzhuorum*. *B. chenghuang* with very few cusps on the outer marginal teeth is almost exclusively found on big rocks, while *B. liangzhuorum* with more cusps on the outer marginal teeth is almost exclusively restricted to soft sandy/muddy substrate. The deep water dweller *B. paradoxa* has more cusps than *B. boettgeri*, which dwells in shallow water in the same river. We consider that more ecological studies should be conducted on *Bakyietaia* to get a better understanding of its radula diversification and adaption.

With seven species including four endemic ones, Hainan Island harbours the highest biodiversity of *Bakyietaia*. According to the COI phylogeny (Fig. 2), three endemic species (*B. fontinalis*, *B. luuemxlong*, *B. naiadica*) form a monophyletic group; two species distributed in central eastern Hainan (*B. fontinalis*, *B. luuemxlong*) are sister species, and the species distributed in northern Hainan (*B. naiadica*) is the sister species of the two central eastern species. Non-adaptive radiation refers to a lineage diversification without obvious ecological diversification, which is common in allopatric or parapatric taxa (Rundell & Price,

2009). With allopatric distributions and similar ecological niche, these three species arguably form a small non-adaptive radiation endemic to Hainan Island. Similar non-adaptive radiation of large freshwater snails in the insular environment can be found from Madagascar (Köhler & Glaubrecht, 2009). Madagascar has a larger species radiation of freshwater snails comparing Hainan, but Hainan is much smaller in area comparing Madagascar (1/20 of Madagascar). In addition, *B. luikongi* endemic to Leizhou Peninsula is the sister species of the entire Hainan clade. This suggests that the repeated Pleistocene flooding of Qiongzhou Strait, the narrow marine strait (minimum width 18 km) separating Leizhou Peninsula and Hainan Island, has caused the separation of the *B. luikongi* and the clade of three Hainan species.

Bakyietaia boettgeri and *Bakyietaia jingweiae* both display a disjunct island (Hainan) - mainland (Guangxi/Vietnam) distribution (Fig.16, Fig. 17, Fig.55). Notably on Hainan Island, *B. boettgeri* is only recorded from the northern region and *B. jingweiae* is only recorded from the western region. We consider that such a distribution pattern reflects the marine regression in the South China Sea. Lin *et al*. (2023)'s study has revealed the similarity of freshwater fish community assemblages of Hainan, Guangxi and parts of the Red River Basin of Northern Vietnam. Zhang *et al*. (2020) identified a paleo-river delta off the southwest coast of Hainan Island around 65 to 56 kyr BP, and both the Red River in Vietnam and the rivers of Hainan contributed to this delta. The reconstructed Red River Basin during the Last Glaciation Maximum connected northern Vietnam, Guangxi and western to northern Hainan (Fig. 55). This paleo-river basin provided an opportunity for the dispersal of freshwater fauna, including the *Bakyietaia* species.

In conclusion, the new freshwater genus *Bakyietaia* is a highly diverse group of Viviparidae confined to South China and mainland southern East Asia. The distribution pattern of *Bakyietaia* species and their differences in morphology and ecology also make it a good model to study speciation, adaption and biogeography in freshwater in this region. Further studies combining systematics, ecology and genomics and more intensive sampling in Vietnam and Guangxi in particular are needed to uncover the evolutionary history of this highly diverse group.

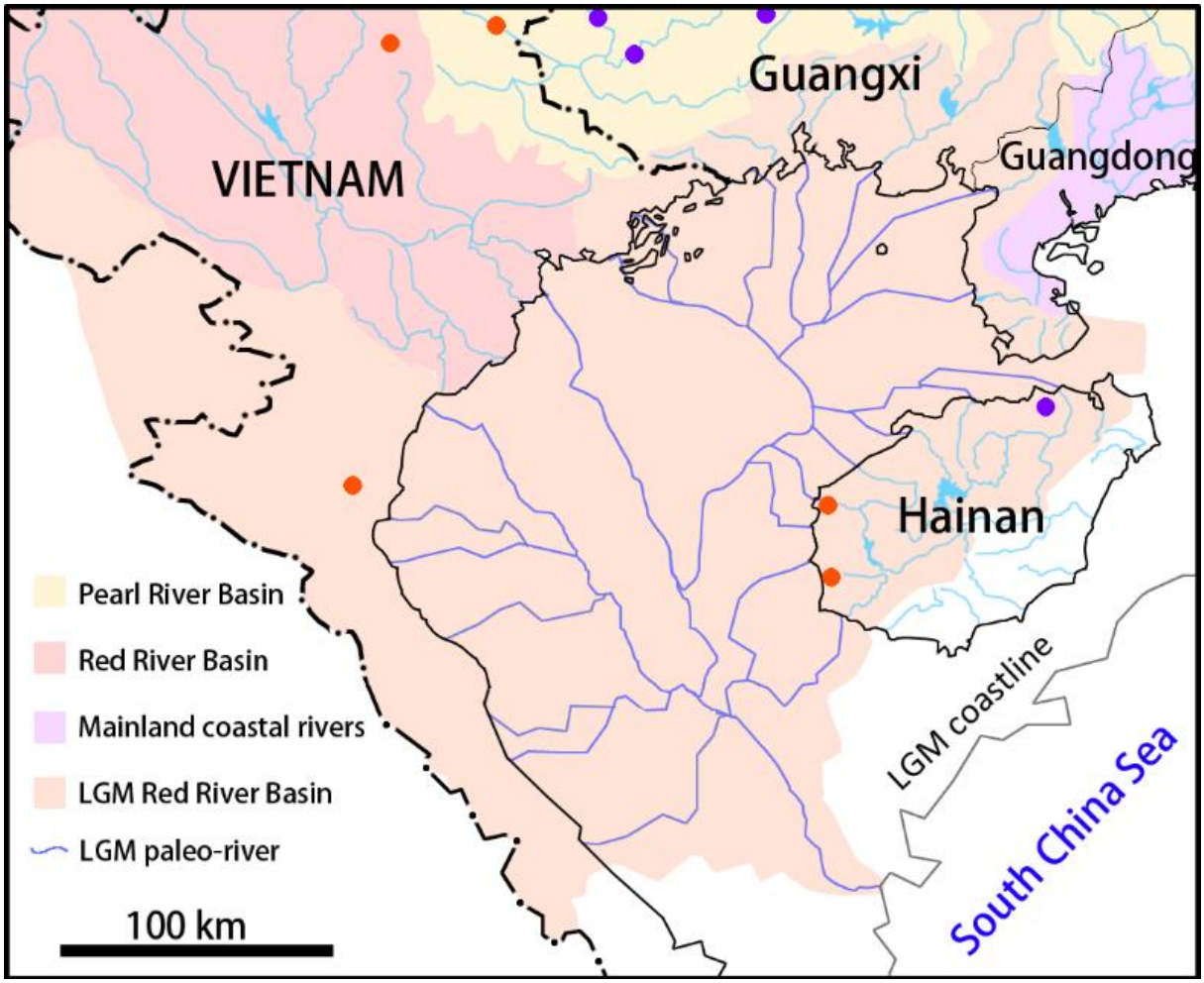


Figure 55. The reconstruction of current and paleo-coastlines, river basins and rivers near Hainan Island and northern Vietnam during the Last Glaciation Maximum (LGM), modified based on Lin *et al*., 2023. The dots refer to the collecting sites of *Bakyietaia boettgeri* (purple) and *Bakyietaia jingweiae* (red).

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Rediscovery and systematics of the enigmatic genus *Helicostoa* reveals a new species of sessile freshwater snail with remarkable sexual dimorphism

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Helicostoa sinensis E. Lamy, 1926 is a unique freshwater gastropod species with a sessile habit. This enigmatic species was first found cemented on river limestones from China about 120 years ago and described together with the genus. It was never collected again and has been considered monotypic. Here, we report the rediscovery of *Helicostoa* from several rivers in China, and describe a second species of this genus based on a comprehensive study. In addition to the unique sessile habit of both species, the new *Helicostoa* species presents one of the most remarkable cases of sexual dimorphism within molluscs. Only the adult female is sessile and the original aperture of the female is sealed by shell matter or rock, while an opening on the body whorl takes the function of the original aperture. The male is vagile, with a normal aperture. Our results confirm the recently suggested placement of *Helicostoa* within the family Bithyniidae. The sessility of *Helicostoa* species is considered as an adaption to the limestone habitat in large rivers. The extreme sexual dimorphism and secondary aperture of females are considered as adaptations to overcome the obstacles for mating and feeding that come with a sessile life style.

1. Introduction

Sexual dimorphism is widespread and common in animals. Sexual selection, fecundity selection and ecological causation are the three popular explanations for the evolutionary mechanism of sexual dimorphism [1]. Within molluscs, sexual dimorphism is mostly reflected in size differences (e.g. in land snails [2], freshwater snails [3] and marine bivalves [4]); only in a few cases is sexual dimorphism found in shell structure (e.g. in marine [5] and freshwater snails [6]); in some rarer cases, such as the cephalopod *Argonauta* or the Puelche oyster [7], sexual dimorphism extends to striking difference in life history.

Helicostoa sinensis is a species of freshwater gastropod described based on about 260 snails cemented to two pieces of limestone collected from China in around 1900. The syntopic sessile mussel *Limnoperna fortunei* (Dunker, 1857) confirmed that they were collected from a freshwater environment [8]. It was assigned to a new family of ' taenioglossate prosobranchs' gastropods, Helicostoidae, based on a study of opercula, dry soft tissue and radulae left in the shell [9]. The shells cemented to these limestones displayed two completely different morphotypes: the large, flat 'P type' and the small, spiral 'T type'. The 'P type'

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is much more numerous on the syntype limestones than the 'T type' (ratio of at least 4 : 1). Pruvot-Fol suggested that these two morphotypes probably represent different sexes of the same species, viz. a case of sexual dimorphism, despite the lack of any anatomical evidence [9]. Bequaert and Clench considered that the radula and soft parts of this species were similar to that of the species of Valvatidae or Bulimidae (today Bithyniidae) [10]. This species was more recently assigned to the sessile marine snail family Vermetidae Rafinesque, 1815 [11] or kept in Helicostoidae within superfamily Truncatelloidea [12].

Helicostoidae was considered as a monotypic family of Mollusca endemic to China. Neither were the two types of *H. sinensis* collected again since their discovery more than 120 years ago, nor was any additional species of this family found. All specimens of this species were collected from a city called 'Kouei-Tchéou' near Yangtze River, more than 1200 km away from Shanghai [8], probably referring to the Three Gorges region [13]. However, no specimen of *H. sinensis* was found during comprehensive surveys of freshwater molluscs in the Three Gorges region of Yangtze River in 1980s [14] and in 1990s [15], which were conducted prior to the construction of the Three Gorges Dam.

Based on a study of historical DNA of the 'P type' syntype, Wilke *et al.* proposed that *H. sinensis* should be a subfamily-level taxon within the family Bithyniidae, and Helicostoidae is a synonym of Bithyniidae [16], thus finally clarifying the phylogenetic position of this species. *H. sinensis* is conservatively classified as 'Critically Endangered' due to the habitat loss in Three Gorges region and no collecting record for more than 120 years.

In March of 2022, we accidentally rediscovered the living 'T type' of this enigmatic sessile freshwater snail in a large tributary of the Pearl River Basin in Guangxi, China, which is far from the Yangtze River (at least 550 km to the main stream). Follow-up surveys led to the discovery of more populations of this species from other tributaries of Pearl River Basin. We conducted a comprehensive study on the morphology, anatomy, phylogeny, and ecology of this species and compared the fresh samples with historic museum collections. Based on molecular phylogeny and shell morphology, we found that 'T type' and 'P type' should be different species of *Helicostoa*, instead of representing different sex morphotypes of one species *H. sinensis* as proposed by Pruvot-Fol [9]. However, we indeed found a remarkable sexual dimorphism in 'T type'. Here, we revise the taxonomy of *Helicostoa* and describe 'T type' as a new species *Helicostoa liuae* sp. nov. based on an integrative taxonomic approach. The evolutionary innovations, sessility, sexual dimorphism and distribution of *Helicostoa* are discussed in this paper.

2. Material and methods

(a) Materials

The specimens were collected in three field trips in Long River, Hechi, Guangxi, China during May, July and October 2022, and bought from a local fisherman near the junction of Qian and Xun River, Guiping, Guangxi, China in September 2022 (figure 1). The snail-carrying limestones were collected by hand from a depth of 0.5 metres in the Long River. The sessile specimens used for this study were peeled off from the limestones and kept in 70% ethanol. Some living animals were collected and kept in aquaria in Beijing and Shanghai for several weeks to months. The holotype (female) and the allotype (one designated male paratype) are kept in the collection of Zoological Museum of China, Institute of Zoology, Chinese Academy of Sciences, Beijing (IZCAS), other paratypes are kept in the collection of Museum of Biology Peking University, Beijing (PKU), Museum für Naturkunde, Berlin (ZMB), Muséum national d'Histoire naturelle, Paris (MNHN) and private collection of Zhe-Yu Chen (CZY) and Zi-ang Shi (SZA).

(b) Examination of morphology

The specimens were photographed in consistent orientation using focus stacking methods with a Sony alpha 7R 4 camera or Canon 5D Mark IV camera attached with a Laowa 25 mm f/2.8 2.5-5X Ultra Macro lens, and a Nikon SMZ18 or Leica M205C stereomicroscope. NIS-Elements D-software and LAS X-software were used to do measurements and focus stacking. The following dimensions were measured on the photos to a precision of 0.1 mm: height (H) and width (W) of shell, height (AH) and width (AW) of aperture, width of opening on body whorl (WO). The WO was measured at the opening (see electronic supplementary material, figure S2*b*). Number of whorls (N) was counted to a precision of ¼ whorls. The protoconch was photographed in detail with Sony alpha 7R 4 camera and the Mitutoyo M-Plan APO 10× Lens. Radulae were extracted through dissection, cleaned by boiling in 1% NaOH solution for half an hour, and rinsed with distilled water. Shell of the attached part, radulae and operculum were coated with gold before scanning electron microscopy (SEM) with FEI Helios Nanolab G3 UC, FEI Quanta 450 FEG or Zeiss *EVO LS10* scanning electronic microscope. The snail bodies, male genitals and egg capsules were taken out during dissection and photographed under a stereomicroscope (Leica MDG34 or Nikon SMZ18). The operculum has been described according to Zhang & von Rintelen [17].

(c) DNA extraction and amplification

DNA was extracted from the animals of altogether seven individuals from Hechi and Guiping, using a CTAB/chloroform extraction or alkaline lysis protocol. Two mitochondrial genes, cytochrome c oxidase subunit I (COI) and 16S rDNA (16S), were targeted, and partial sequences were amplified through polymerase chain reaction (PCR) with the use of the following primer pairs: LCO1490, 5′-GGTCAACAAATCATAAAGATATTGG-3′ [18] and COX-B7R, 5′-ACCACCAGCTGGATCAAAAA-3′ [19] for COI; 16Sar-L, 5′-CGCCTGTTTATCAAAAACAT-3′ and 16Sbr-H, 5′-CCGGTCTGAACTCAGATCACGT-3′ [20], for 16S . PCR amplifications were conducted in volumes of 25 µL under the following cycling conditions: initial denaturing step at 95°C for 2 min (for COI) or for 3 min (for 16S), followed by 35 cycles of 95°C for 45 s (for COI) or 30 s (for 16S), 57°C for 45 s (for COI) or 52°C for 30 s (for 16S), and 72°C for 30 s, with a final extension step of 10 min at 72°C. Sequencing was conducted by Macrogen Europe, Amsterdam, Netherlands and Tsingke Biotechnology, Beijing, China.

(d) Phylogenetic analysis

Altogether 14 sequences (7 for each gene) of *H. liuae* have been uploaded into Genbank. The dataset was complemented by two sequences of *H. sinensis* from the historic specimens from Wilke *et al.* [16] and 26 additional sequences that were selected based on the phylogeny from Wilke *et al.* [16] and Bunchom *et al.* [21] (electronic supplementary material, table S1). Sequences were aligned using the Muscle algorithm [22] as implemented in Geneious Prime 2020 (https://www.geneious.com). The genetic distances were calculated using MEGA X [23]. The data

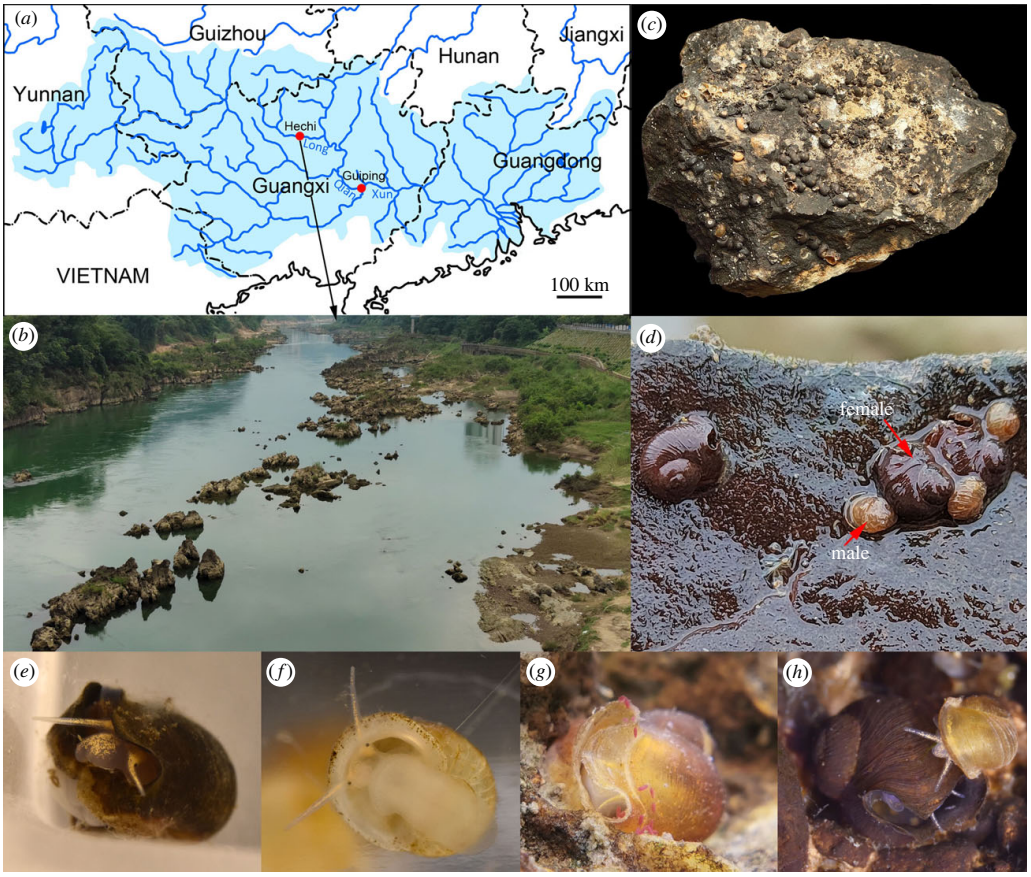


Figure 1. Specimen collecting sites in the Pearl River Basin of China, habitat and living animals in the aquarium for *Helicostoa liuae* sp. nov. (*a*) Map of the Pearl River Basin (light blue area) and the localities of two collection sites (red dot), Hechi (type locality) and Guiping in Guangxi, China. (*b*) Environment of the type locality in Long River, Yizhou, Hechi City, with many large limestones in the river. (*c*) The limestone where the holotype was collected. (*d*) The living animals of females and males *in situ* on the limestone just taken from the river. (*e*) A female detached from stone. (*f*) A vagile male. (*g*) A sessile female in excretion after feeding on red *Chlamydomonas reinhardtii* algae, photographed by Min Chen. (*h*) A male climbing on a female, displaying the remarkable difference in size.

set was tested in MEGA X for the best-fit model of sequence evolution by means of the Akaike and Bayesian information criteria. GTR + G + I (for COI) and GTR + G (for 16S) were suggested as the best-fitting nucleotide substitution models, which were employed in the phylogenetic analyses using Maximum likelihood (ML) and Bayesian inference (BI). A ML analysis was conducted using RAxML [24] as implemented in Geneious Prime2020 with 1000 bootstrap replicates. The BI analysis was conducted using MrBayes 3.2.6 [25] as implemented in Geneious Prime 2020 with four independent chains for 5 000 000 generations, samplefreq = 1000, and burnin = 25%.

3. Results

(a) Genetic differentiation and phylogeny

The p-distance of COI between *H. liuae* and *H. sinensis* is approximately 2.3% to 2.4%; the p-distance of COI within *H. liuae* is approximately 0 to 0.8%. The p-distance of 16S between *H. liuae* and *H. sinensis* is approximately 2.1%; the p-distance of 16S within *H. liuae* is approximately 0 to 0.4%.

The phylogenetic trees reconstructed by BI and ML are highly congruent in topology, therefore only the BI tree is shown (figure 2). The molecular phylogeny places *Helicostoa* within the family Bithyniidae with high support (figure 2). All sequenced individuals of *H. liuae*, irrespective of sex or geography, form a highly supported clade. *Helicostoa* is monophyletic and *H. liuae* is the sister species of *H. sinensis*.

(b) Scanning electron microscopy of the attached part

The attached part of *H. liuae*, which should be the original aperture of shell, is sealed by shell matter or rock. Scanning electron micrograph images of the attached part revealed that many foreign objects were embedded into the shell during the development and cementation of the snail (electronic supplementary material, figure S1). On the surface of the attached part of the shell of specimen 1, many long micro-tubes were found clustering together (electronic supplementary material, figure S1*a–i*). The micro-tubes seemed soft and smooth. The diameters of micro-tubes were all about 5 µm and their length reached 50 to 60 µm. The cross section of the micro-tube (electronic supplementary material, figure S1*e*) and area with micro-tubes (electronic supplementary material, figure S1*f–g*) showed that these tubes were embedded into the shell, which displayed a different

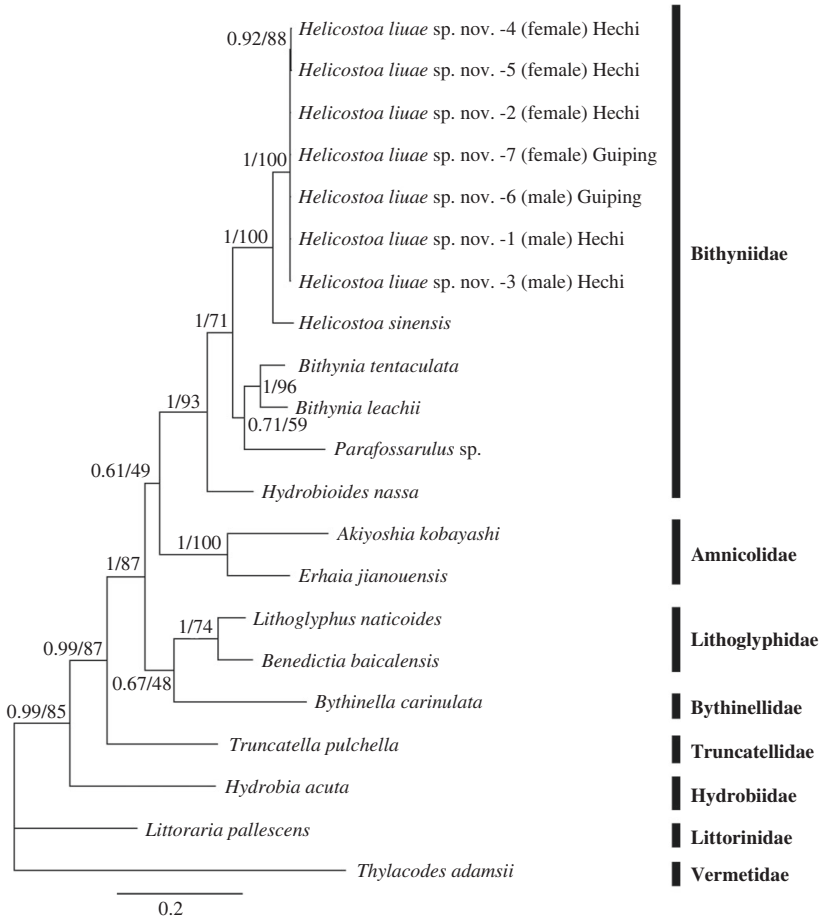


Figure 2. The BI tree showing the phylogenetic position of genus *Helicostoa* within Bithyniidae based on concatenated sequences of cytochrome c oxidase subunit I (COI) and 16S rDNA. Numbers above branches are BI posterior probabilities/ML bootstrap values.

microstructure in comparison to areas of the shell without foreign objects (electronic supplementary material figure S1*h*). On the surface of the attached part shell of specimen 2, no micro-tube like those on specimen 1 were present, but many irregular particles (electronic supplementary material, figure S1*j–l*). Similar irregular particles were also observed from the attached parts of two additional specimens.

(c) Systematics

Superfamily Truncatelloidea Gray, 1840

Family Bithyniidae Gray, 1857

***Helicostoa* E. Lamy, 1926**

Type species: *Helicostoa sinensis* E. Lamy, 1926, by original designation.

Diagnosis: shell 2.68 to 11.80 mm in width, broad protoconch, 2.5 to 4 whorls, teleoconch whorls with dense fine ribs, for the sessile individuals on the middle of the last half whorl upper end and lower end of outer lip extending and merging together, enclosing an opening, seam between both ends of outer lip clearly visible.

Taxonomic remarks: *Helicostoa sinensis* used to be considered as the only species of the genus *Helicostoa*, which was assigned to the monotypic family Helicostoidae Pruvot-Fol, 1937. However, both Wilke *et al*. [16] and our molecular phylogeny support a position of *Helicostoa* within Bithyniidae, and Helicostoidae must consequently be regarded as a junior synonym of Bithyniidae. Our study of operculum, radula and genital characters supports this taxonomic revision as well. There are two known species of *Helicostoa*. The description of *Helicostoa sinensis* is a redescription incorporating our new results.

***Helicostoa sinensis* E. Lamy, 1926**

Lectotype: MNHN-IM-2000-33309, 'P type', 'Koué-Tchéou, ville située sur le Yang Tsé Kiang, à plus de 1200 kms de Chang-Hai'.

Paralectotypes: MNHN-IM-2000-38619, 204 specimens of 'P type', on the same limestone of the lectotype; MNHN-IM-2000-38620, 60 specimens of 'P type'.

Description: shell (figure 3, table 1) 9.30 to 11.80 mm in width, discoidal, orange yellow to light red in colour; usually with 3 inflated whorls, protoconch broad smooth, teleoconch whorls with dense fine ribs, with one obvious keel on the upper part of second whorl; body whorl turning upward, covering spiral whorls partly, and turning downward to attach the substrate, sometimes open-coiled due to obstacle,

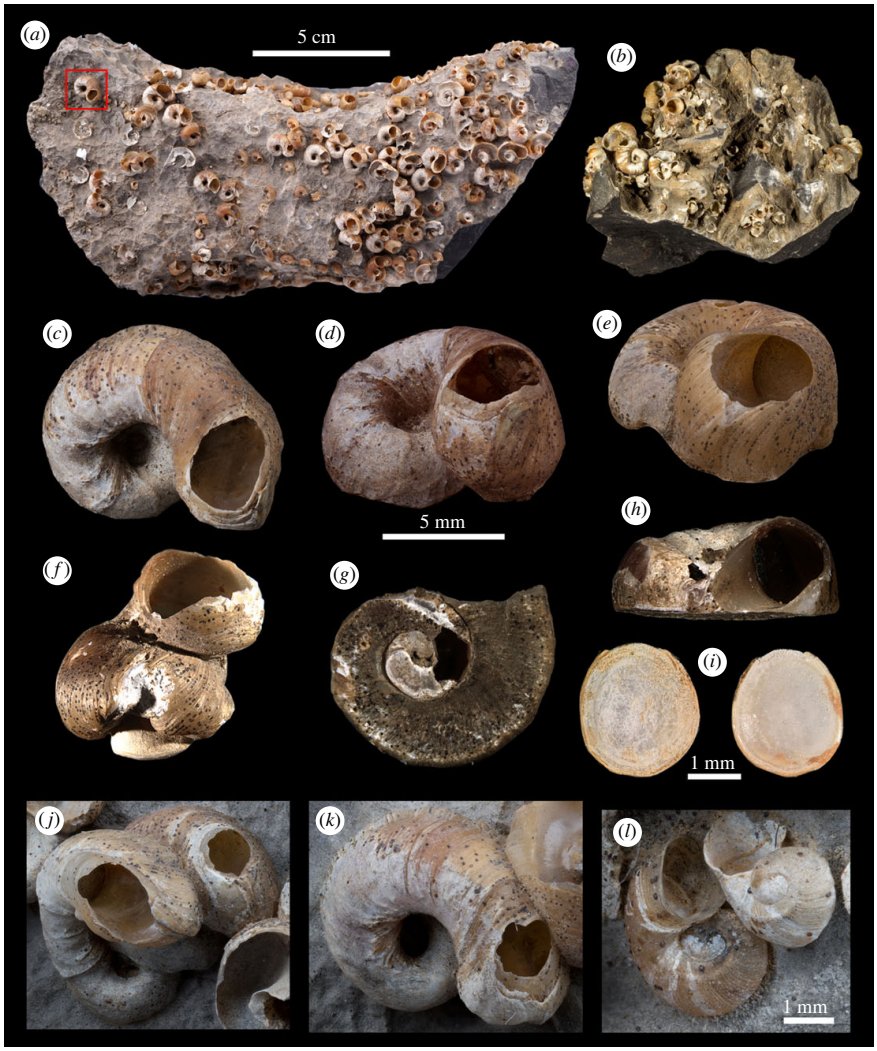


Figure 3. Types of *Helicostoa sinensis* E. Lamy, 1926. (*a*) The stone bearing specimens; lectotype MNHN-IM-2000-33309 is in the red frame. (*b*) The stone bearing specimens; MNHN-IM-2000-38620 (credit: MNHN-Manuel Caballer 2017). (*c–d*) Details of MNHN-IM-2000-33309, lectotype. (*e*) A paralectotypes showing the details of seam under the opening. (*f*) An open-coiled paralectotype. (*g*) The base of a peeled paralectotype showing the sessile part. (*h*) The side view of a paralectotype showing the flat base. (*i*) Operculum, left is exterior surface and right is interior surface. (*j–k*) paralectotypes *in situ* on rock. (*l*) Juveniles showing the protoconch. (*a*) Scale bar 5 cm; (*c–h*) scale bars 5 mm; (*i*) scale bar 1 mm; (*l*) scale bar 1 mm.

Table 1. Shell measurements of *Helicostoa* species. Values are arithmetic mean (mm) followed by standard deviation except for number of whorls. Males of *H. liuae* do not have the opening. Aperture of *H. sinensis* is not visible and openings of *H. sinensis* are mostly broken. Therefore, WO of *H. liuae*, AH, AW and WO of *H. sinensis* are not recorded.

species	gender	H	W	AH	AW	WO	W/H
H. liuae ($n = 24$)	female	4.71 ± 0.41	5.08 ± 0.52	2.61 ± 0.48	3.45 ± 0.40	0.66 ± 0.10	1.08 ± 0.08
H. liuae ($n = 10$)	male	1.96 ± 0.65	2.70 ± 0.84	1.49 ± 0.51	1.71 ± 0.58	/	1.38 ± 0.08
H. sinensis ($n = 10$)	unknown	5.14 ± 0.64	10.52 ± 0.89	/	/	/	2.06 ± 0.20

the edge of body whorl attaching the rock sometimes extended, forming a narrow flat ridge, the area attaching the substrate flat; on the middle of the last half whorl upper end and lower end of outer lip extending and merging together, enclosing a large round or oval opening, seam between both ends of outer lip clearly visible; original aperture completely sealed by shell and attached rock.

Operculum (figure 3*i*) calcareous, nearly round, thin, transparent white in colour; on exterior surface the nucleus not clearly visible, with many scales on central part and

concentric growth line on outer region; on interior surface a large inner opercular region visible.

Morphological comparisons: see in *Helicostoa liuae* sp. nov.

Ecology: this species was only found on the surface of limestones in rivers.

Distribution: this species was only known from the type locality 'Koué-Tchéou, ville située sur le Yang Tsé Kiang, à plus de 1200 kms de Chang-Hai', viz. 'Kouei-Tchéou, city located on the Yangtze River, more than 1200 km away from Shanghai.' 'Kouei-Tchéou' in French may refer to '夔州' (Kuizhou, current Fengjie County near Three Gorges region) or '贵州' (Guizhou Province).

***Helicostoa liuae* Zhang, Shi & Chen sp. nov.**

LSID urn:lsid:zoobank.org:act:07CE74EC-AEC9-4A54-8883-DCED59B513A6

Holotype: adult female (IZCAS-FG-609823) collected on 8 May 2022 by Xu-Cheng Wei from limestone in Long River, under Long River Bridge, Yizhou District, Hechi City, Guangxi Zhuang Autonomous Region, China.

Paratypes: 1 adult male designated as allotype (IZCAS-FG-609824), 16 adult females (5 IZCAS, 5 PKU, 3 SZA, 3 CZY), 17 adult males (7 IZCAS, 8 PKU, 1 SZA, 1 CZY), 1 limestone with 9 adult females and 7 adult males (IZCAS), 1 sub-adult male (CZY), 8 sub-adult females (3 IZCAS, 5 CZY), all collected on July 18, 2022 by Zi-Ang Shi from limestone in Long River, Yizhou District, Hechi City, Guangxi Zhuang Autonomous Region, China (24.5020206°N, 108.6306600°E); 3 adult females (ZMB), 4 adult females (CZY), all collected on May 8, 2022 by Xu-Cheng Wei from the limestone in Long River, Yizhou District, Hechi City, Guangxi Zhuang Autonomous Region, China; 6 adult females (4 IZCAS, 2 PKU), 3 adult male (1 IZCAS, 2 CZY), 1 adult female (CZY), all collected by local people in the junction of Qian, Yu and Xun River in Guiping City, Guangxi Zhuang Autonomous Region, China; 1 adult female (MNHN-IM-2000-38621, 'T type'), 15 adult females (MNHN-IM-2000-38622, 'T type').

Etymology: the species is named in honour of Yue-Ying Liu, the late pioneer researcher of Chinese freshwater molluscs.

Description: shell of adult female (figure 4*a–f*; electronic supplementary material, figure S2*a–f*; table 1) 4.17 to 6.75 mm in width, globose, thin but solid, orange yellow to red in colour; low spiral, most with around 3.5 to 4 inflated whorls, protoconch broad and smooth, teleoconch whorls with dense fine ribs; on the middle of the last half whorl upper end and lower end of outer lip extending and merging together, enclosing a wide bow-shaped opening, convex edge of the bow-shaped opening close to the original aperture, seam between both ends of outer lip clearly visible; original aperture large, ovate, but sealed by shell and attached rock, foreign objects sometimes embedded into sealed aperture, inner lip extending and covering umbilicus.

Shell of adult male (figure 4*g–l*; electronic supplementary material, figure S2*g–n*; table 1) 2.61 to 3.51 mm in width, oblate, thin but solid, pale yellow to orange yellow in colour; low spiral, most with 2.25 to 3 inflated whorls, protoconch broad and smooth, teleoconch whorls with dense fine ribs; aperture large, ovate, labral margin obviously extended, umbilicus mostly covered by inner lip, sometimes open.

Protoconch (figure 5*h*) broad, low dome-shaped, nucleus of protoconch relatively smooth, the rest of protoconch with fine granule and several spiral striations; no other obvious sexual dimorphism except for the size.

Operculum (figure 5*a–b*) calcareous, ovate, thin and fragile, transparent white in colour; on exterior surface a paucispiral nucleus visible, with one anti-clockwise whorl, located on the lower central part, concentric growth lines surrounding the nucleus; on interior surface a large inner opercular region visible, with many grains and veins; no other obvious sexual dimorphism except for the size.

Head, foot and mantle of living animals (figure 5*c*) transparent white or grey, with fine dense yellow pigment spots on the upper side of snout, tentacles, region around eyes and mantle edge, and sparse yellow pigment spots and dense fine black spots on the mantle; ethanol-preserved specimens (figure 5*d–e*) with pale yellow body colour and irregular black spots on the surface of mantle.

Male genital (figure 5*g*) with very long flagellum, about five times as the length of penis; the thickness of penis uniform, tip pointed, length of penial appendix same as that of distal part (from the base of penial appendix to the tip of penis) of penis, length of distal part of penis same as that of proximal part (from the base of penial appendix to the base of penis) of the penis; penis situated in the neck behind the right tentacle of male.

Egg capsules kept in ethanol (figure 5*f*) white, with a group size of 6 individuals, clustering together tightly into a round flat shape.

Radula ($n = 5$, 3 females and 2 males; electronic supplementary material, figure S3) oblong. Central teeth with 5 to 6 cusps in females or 4 cusps in males on either side of median cusp, median cusp with wider base longer than adjacent cusps; 3 to 4 cusps on either lateral edge of central teeth, inner pair much larger than others. Lateral teeth with 5 to 7 cusps in females or 5 to 6 cusps in males on either side of median cusp, median cusp with wider base longer than adjacent cusps; inner lateral edge of lateral teeth with a concave on lower part, only half of outer lateral edge in length. Marginal teeth slender, slightly oblong; inner marginal teeth with 25 to 41 cusps in females or 29 to 43 cusps in males, the fourth or fifth cusp in females or sixth to seventh cusp in males counting from outer side larger than the other cusps; outer marginal teeth with 23 to 24 cusps in females or 23 to 27 cusps in males.

Morphological comparisons: the shell of *Helicostoa liuae* sp. nov. displays a striking sexual dimorphism, which is related to different life histories of males and females. The shell of the sessile adult female can be distinguished from that of the vagile adult male based on its much larger size, more whorls, higher spiral, closed primary aperture, and with a bow-shaped opening. The sessile adult female can be distinguished from *Helicostoa sinensis* based on its much smaller size, conical shell, a wide bow-shaped opening and ovate operculum. The shell of vagile adult male is most similar to that of another bithyniid species *Sierraia expansilabrum* Brown, 1988 from Sierra Leone, which has a small globose shell and extended labral margin. However, it can be easily distinguished from species of *Sierraia* based on its much smaller oblate orange shell, operculum characters and many more cusps on the marginal teeth of radula. The shell of juvenile female of *Helicostoa liuae* sp. nov. can be distinguished from that of the juvenile male based on its higher spire.

Ecology: this species is mainly found on the surface of limestone rocks in big rivers, but it sometime also can be found attached on bricks and snail or mussel shells in big

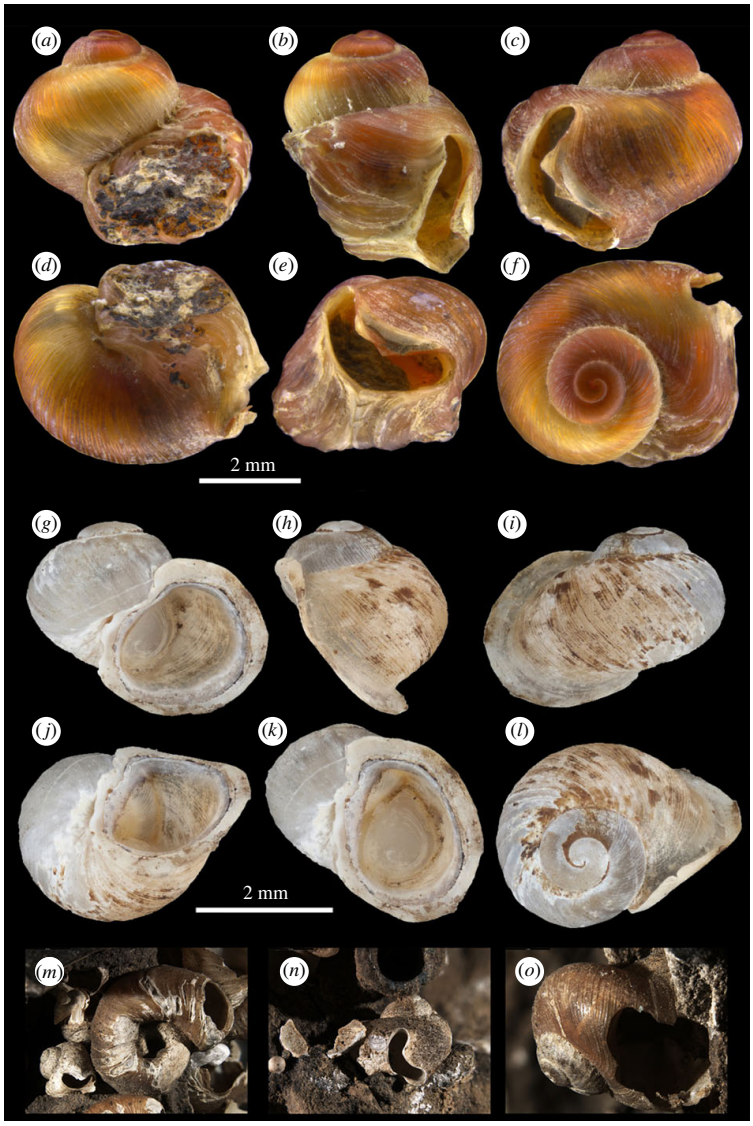


Figure 4. Shell of *Helicostoa liuae* sp. nov. (*a–f*) Holotype, IZCAS-FG-609823, adult female, with same scale bar 2 mm. (*g–l*) Allotype, IZCAS-FG-609824, adult male, with same scale bar 2 mm. (*m–o*) Paratypes *in situ*, MNHN-IM-2000-38622, adult females, (*m*) showing the coexisting of two *Helicostoa* species on one rock.

rivers; adult females are sessile and adult males are vagile (figure 1*f*); sessile adult females produce egg capsules in the cavity of shell, and capsules cluster together, tightly attaching to the sealed aperture. When kept in aquarium, the sessile females could actively do suspension feeding; the red form of living planktonic algae *Chlamydomonas reinhardtii* was accepted as food by the snails, and the snails excrete red faeces (figure 1*g*); the sessile females could stretch out from the secondary aperture (figure 1*e*), but normally only stay close to the secondary aperture in the shell (figure 1*g*).

Distribution: the species currently is only found in Long River (龙江), Qian River (黔江) and Xun River (浔江) in Guangxi Zhuang Autonomous Region, China. However, since this species was found syntopic with *Helicostoa sinensis*, it apparently shares or used to share at least parts of its distribution range with *Helicostoa sinensis*.

4. Discussion

(a) Taxonomy and evolutionary innovation

In this study, we rediscovered the 'T type' of enigmatic sessile freshwater snail *Helicostoa* lost for a century. In agreement with the results of Wilke *et al*. [16], our phylogeny based on COI and 16S supports the assignment of genus *Helicostoa* to the family Bithyniidae. Therefore, the family Helicostoidae is a junior synonym of Bithyniidae. In addition, morphological and anatomical characters also strongly support that *Helicostoa* is a memeber of Bithyniidae. The structure of the male genital (figure 5*g*) is quite similar to that of *Bithynia* [26]; the radula (electronic supplementary material, figure S3) displays a typical structure found in Bithyniidae; the calcified operculum (figure 3*i*; figure 5*a–b*) is also typical for Bithyniidae [27–29]. The subfamily-level taxonomy of

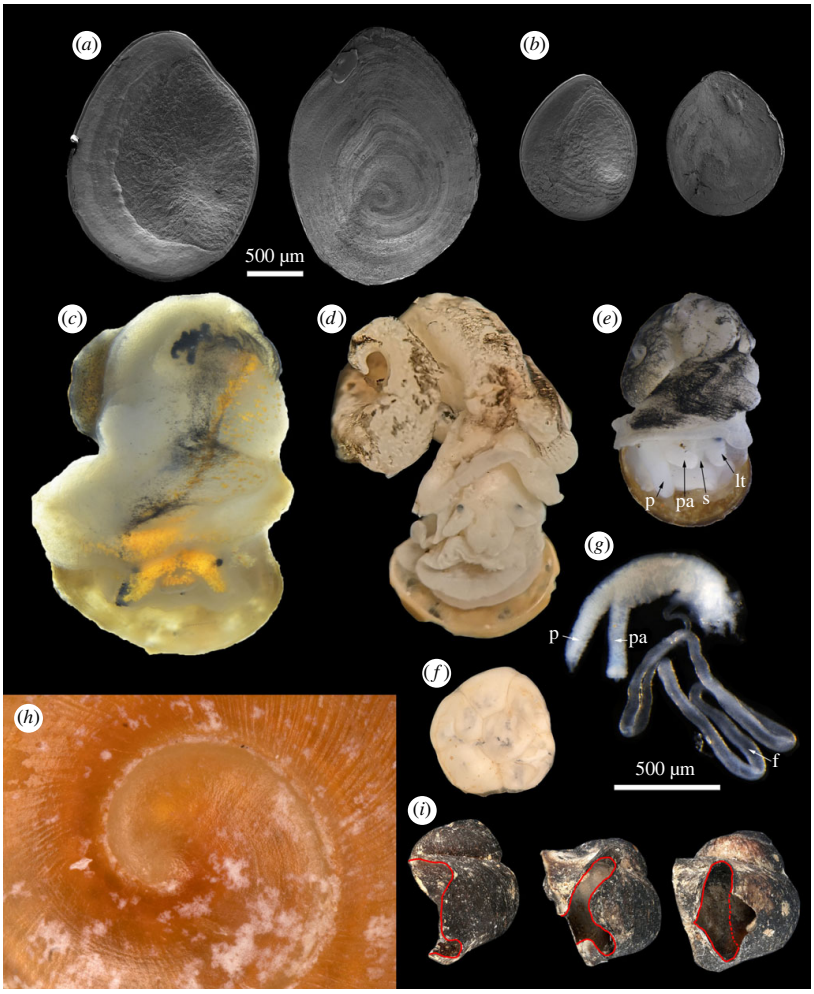


Figure 5. Morphological details of *Helicostoa liuae* sp. nov. (*a*) SEM photo of the female operculum, left is interior surface and right is exterior surface. (*b*) SEM photo of the male operculum, left is interior surface and right is exterior surface. (*c*) Fresh soft body of female. (*d*) Ethanol preserved soft body of female. (*e*) Ethanol preserved soft body of male. (*f*) Egg capsules. (*g*) Male genital. (*h*) Protoconch of female, photographed by Bernhard Schurian. (*i*) Different stages of sub-adult females showing the enclosing process of the opening. (*a–f*) Scale bar 500 µm; (*g*) scale bar 500 µm. Abbreviations: p, penis; pa, penial appendix; s, snout; lt, left tentacle; f, flagellum.

Helicostoa within the new subfamily Helicostoinae proposed by Wilke *et al*. [16] should be revised based on a more integrative study of Bithyniidae involving more genera and species in the future.

The molecular phylogeny strongly suggests that the 'T type' should be a different species from the 'P type'. The 'P type' now is designated as *H. sinensis*, and the 'T type' is described as a new species *H. liuae*. The p-distance of COI and 16S between *H. sinensis* and *H. liuae* support that they should be two different species within one genus. No genetic differences were found between two sequenced populations of *H. liuae* that are geographically far apart. As for morphological characters, *H. sinensis* can be easily distinguished from both genders of *H. liuae* based on its discoidal shape, much larger size, round opening and round operculum, which is consistent with the results of Lamy [8] and Pruvot-Fol [9]. We did not find a jaw in specimens of *H. liuae*, which was mentioned by Pruvot-Fol [9] for the 'T type'. Since a jaw has never been observed in Bithyniidae, we suspect that Pruvot-Fol misidentified this character.

The sessile individuals of *Helicostoa* species cannot be confused with any other species of superfamily Truncatelloidea, especially when considering the opening on the body whorl. The opening on the body whorl of sessile *H. liuae* is wide bow-shaped, and that of *H. sinensis* is round. The growing process of this opening can be seen in several juvenile shells (figure 5*i*). Based on aquarium observations, the opening of *H. liuae* take over the function of the aperture sealed by shell matter and stone. Sessile *H. liuae* could stretch their heads or tentacles out, secret mucus, do ingestion and excretion from this secondary aperture (figure 1*e*,*g*). Although we have not observed the living *H. sinensis*, we suspect that the opening of sessile *H. sinensis* should play the same role as that of *H. liuae*.

The morphology of this opening in *Helicostoa* is somehow similar to that of selenizone in a few marine snail species of

Scissurelloidea Gray, 1847, such as *Satondella danieli* Segers, Swinnen & Abreu, 2009. The selenizone of Scissurelloidea is a slit or a single opening on the body whorl, with the main function of respiration [30]. However, this similarity is obviously the result of convergent evolution, since there is no close relationship between Bithyniidae of Caenogastropoda and Scissurelloidea of Vetigastropoda. The single opening on the body whorl working as the aperture seems like a unique evolutionary innovation of *Helicostoa* within entire Mollusca.

(b) Sessility and sexual dimorphism

Sessility is an extremely rare character within freshwater gastropods. Except for species of *Helicostoa*, *Melanoides agglutinans* is the only other known freshwater gastropod species with a sessile habit. *M. agglutinans* was only found in crevices of rocks immersed in swift water from lower Congo River [31]. *M. agglutinans* seems to adhere foreign objects at any part of its shell where the growing is impeded, and becomes deformed at the sessile part. Bequaert & Clench [31] considered that the deformation and sessility of this species would be probably lost if the young snails were moved from crevices to a less restricted environment, and 'normal' adult shells might be obtained. Besides, there is no sexual dimorphism observed in shell morphology and sessility within this species.

Species of *Helicostoa* display a completely different strategy of sessility from *Melanoides agglutinans*, as sessile females of both *H. liuae* and *H. sinensis* have a specific attachment area instead of attaching randomly to the substrate as *Melanoides agglutinans*. However, the specific attachment areas of the two *Helicostoa* species are different. *H. liuae* attaches itself only at the sealed aperture of the last whorl (figure 4*a*), while *H. sinensis* fixes itself on stones tightly from the base of the second whorls (figure 3*g*) to the sealed aperture. As for *H. sinensis*, the area attaching to the substrate can be quite flat (figure 3*h*), and the body whorl can become open-coiled when it meets an obstacle (figure 3*f*). This difference of attachment area suggests that *H. sinensis* should have an earlier and longer sessile period in life history compared to *H. liuae*. The SEM images of the sessile area of *H. liuae* show that the shell incorporates foreign objects into shell matter, such as micro-tube (electronic supplementary material, figure S1*b–g*) or irregular particles (electronic supplementary material, figure S1j–l). The micro-tubes with their soft, smooth wall and uniform diameter are probably not a structure of the shell but some microorganism like Cyanobacteria.

Sessility in females of *Helicostoa* might arguably be linked to reproduction and have evolved as a form of maternal care, as the females are also brooding (i.e. retaining the eggs within their shells). It might be speculated that this prevents eggs from being swept away by the swift water flow in the habitat of *Helicostoa*. However, egg capsules of other gastropods (*Hua* spp. of family Semisulcospiridae) have been observed on rocks in the same habitats. Moreover, bithyniid species of *Sierraia* endemic to Sierra Leone dwell in a similar habitat on the rock surface in rapidly flowing rivers, but they can move freely and lay their eggs directly onto the surface of other shells [27]. Therefore, there is no clear evidence to support this hypothesis at present. More detailed ecological studies of living animals are necessary to understand its adaption of sessility.

In addition, we discovered a remarkable sexual dimorphism in *H. liuae*. We surprisingly found that all sessile individuals of *H. liuae* are females. Some free-moving micro snails from the same locality were shown to be the males of *H. liuae* based on DNA sequences (figure 2) and characters of the radula (electronic supplementary material, figure S3). The differences of morphology and habits between males and females of *H. liuae* are so significant that we initially considered them to be different species. *H. liuae* displays its remarkable sexual dimorphism in shell size, opening structure on the body whorl, and habit of sessility. The female of *H. liuae* is about two to three times larger than the male (table 1 W, H), and the male is much flatter than the female (table 1 W/H).

The larger size of females can be explained by fecundity selection, especially given that eggs are retained in the shell. However, the driving mechanism of sexual dimorphism in opening structure and habit of sessility is more complicated. The opening structure of sessile females, which has been discussed above, is probably an evolutionary innovation triggered by sessility, since the original aperture is sealed to fix the shell to the substrate. The vagile males can easily search for and copulate with different sessile females. Pruvot-Fol also considered that vagile males would be advantageous for mating [9], where sessility is an obvious disadvantage. The marine sessile gastropod *Vermicularia spirata* (Philippi, 1836) is a protandrous hermaphrodite [32]. The free-living small males can search for and mate with sessile large females, which were males before completing sex reversal. Within Truncatelloidea, protandrous hermaphroditism is only reported from one marine species of the family Vitrinellidae [33]. We consider that *Helicostoa liuae* has evolved an extreme sexual dimorphism instead of protandrous hermaphroditism to overcome the obstacles in mating imposed by sessility. Both the secondary aperture and sexual dimorphism seem to be adaptions to its sessile life history. More studies, including the systematic revision of family Bithyniidae to find the sister group of *Helicostoa*, and ecological study of *Helicostoa*, can help us to better understand the evolution of such a remarkable sexual dimorphism.

As for *H. sinensis*, we strongly suspect that it should have a similarly striking sexual dimorphism. All the sessile individuals of *H. sinensis* are probably females as those of *H. liuae*. However, this assumption can only be tested by finding living *H. sinensis*.

(c) Habitat and distribution

With the discovery of the new *Helicostoa* species, three species of sessile freshwater gastropods are known to science. All of them are attached to rocks in large rivers: Congo River for *M. agglutinans*, Yangtze River (doubtful, see following) for *H. sinensis*, and Pearl River for *H. liuae*.

H. liuae is discovered in three large tributaries of the Pearl River Basin: Long, Qian and Xun River. Qian and Xun River are the main arms of the Pearl River. The average flow of Xun River is 7271 $m^3 s^{-1}$, and the largest flow of it can reach 44 900 $m^3 s^{-1}$ [34]. The type locality of *H. liuae* in Long River has a much lower average flow, about 387 $m^3 s^{-1}$, but its largest flow can also reach 10 400 $m^3 s^{-1}$ [35]. Long River is the upstream river of Liu River, and Liu River is the upstream river of Qian River. The distance between the two collecting localities in Long and Qian along the river course is more

than 300 km. In contrast to *M. agglutinans* inhabiting crevices, the two *Helicostoa* species both dwell on the rock surface. These limestone rocks in big rivers seem to offer a special niche for *Helicostoa*. Although we found some other species of Pomatiopsidae on the rocks, this sessile *Helicostoa* species is undoubtedly the dominant species and shows a special adaptation to this habitat.

Since we did not discover any living or dead *H. sinensis*, the distribution of *H. sinensis* remains enigmatic. However, the coexisting of *H. sinensis* and *H. liuae* on the same type-bearing rocks kept in Paris has already demonstrated that the distributions of two species must overlap to some degree. The distribution of *H. liuae*, the Pearl River Basin, is far from the main arm of Yangtze River. The two river basins are only artificially connected by a canal called Lingqu built during the Qin Dynasty of China (219 BCE). The type locality information of *H. sinensis*, 'Kouei-Tchéou' in French, was based on an around 20 years' old note, not directly recorded by Lamy [8]. It could refer to '夔州' (Kuizhou, current Fengjie County near Three Gorges region) or '贵州' (Guizhou Province).

The Three Gorges region fits the description of 'more than 1200 km away from Shanghai'. However, neither living animals or dead shells of *H. sinensis* have ever been recorded from Yangtze River in the Three Gorges region since the description, even though there have been several comprehensive surveys of freshwater molluscs conducted by experts before the construction of the Three Gorges Dam [14,15]. Since *H. sinensis* seems like an abundant or even dominant species fixed to the rock based on the types (figure 3*a*,*b*), it is strange that not even one empty shell has ever been discovered, if this species was really distributed there. In comparison, we discovered large amounts of empty shells of *H. liuae* on the bank of river at the type locality.

Meanwhile, the Long River, where the type locality of *H. liuae* is located, originates from Guizhou Province (figure 1*a*). It is possible to find *H. liuae* in Guizhou as well. Besides, the Yangtze River Basin and the Pearl River Basin are adjacent in Guizhou Province. Considering the sympatry of two *Helicostoa* species, we strongly suspect that the rocks with the types of *H. sinensis* were collected from a river in Guizhou Province. To raise the possibility finding *H. sinensis*, we suggest that more surveys should be conducted on the rocks from rivers in Guizhou Province in the future.

Freshwater fauna is easily impacted by human activities, such as overharvesting, pollution, transportation, sand mining, damming and riverbank construction. Freshwater gastropods represent some of the most threatened species, with nearly 20% of recorded extinctions within molluscs according to IUCN [36]. Many freshwater gastropods from SW China are facing dramatic population declines [37,38]. *H. liuae* is a relatively common species at the collecting sites in the Long, Qian and Xun River. However, all of these sites are very close to urban area. Many dams have been constructed near the habitats of *H. liuae*, such as the Datengxia Hydropower Station in the Qian River [39]. These factors add high environmental risks to the habitat of this sessile species. Considering the two populations without genetic difference are more than 300 km far apart, *H. liuae* should have a relatively strong dispersal capability in the rivers. More surveys should be conducted in the connecting Liu, Hongshui and Yu River to investigate whether more populations of *H. liuae* exist and to what degree they are continuous.

Ethics. Our work is based on the specimens of an unprotected snail species from a non-protected area in China. Therefore our Chinese colleagues can collecte specimens without a certain permit. The type specimens are mostly deposited in China and some paratypes are deposited in Germany and France. The information of specimens are all in the manuscript.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material [40].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. L.-J.Z.: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, validation, visualization, writing—original draft, writing—review and editing; Z.-A.S.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, validation, visualization, writing—review and editing; Z.-Y.C.: data curation, formal analysis, visualization, writing—review and editing; T.v.R.: formal analysis, supervision, writing—original draft; W.Z.: funding acquisition, methodology, supervision, writing—review and editing; Z.-J.L.: data curation, resources, visualization.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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Chapter 6. Zhang LJ, Bernardes SC, Meng K & von Rintelen T. A new monospecific family of freshwater snail from China with Cretaceous Tethys origin. Manuscript.

A new monospecific family of freshwater snail from China with Cretaceous Tethys origin

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Abstract

An extant monospecific family usually represents a relatively ancient evolutionary lineage with only one living species. Such a species can provide us important insights into a unique evolutionary history of deep time. Based on the materials from a freshwater ancient lake in Yunnan, Southwest China, here we report a remarkable extant gastropod species, *Squamapex taiji* gen. et sp. nov., within a new family of Truncatelloidea, Squamapicidae fam. nov.. The fossil-calibrated phylogeny based on mitochondrial and nuclear loci shows that this monospecific family belongs to a distinct ancient lineage with Cretaceous origin. The two-stage ornamented protoconch with distinct microstructure from the teleoconch of this species is the first known case within true freshwater gastropods. This type of protoconch formerly is only known from marine or migratory gastropods with veliger larvae. The sister group, the unique protoconch and the biogeography indicate a high possibility of Tethys Ocean origin of this new family.

Keywords: ancient Lake, Squamapicidae fam. nov., systematics, taxonomy, Truncatelloidea, Yunnan

Introduction

Truncatelloidea Gray, 1840 is one of the largest and most diverse superfamilies within Mollusca, including 35 families, 715 genera, and at least 6739 accepted species (by March 1st, 2023) [1]. Members of this superfamily are distributed across all oceans and continents, with the exception of Antarctica. They exhibit an extremely broad range of adaptions to various habitats, including the bathyal zone, marine, brackish, freshwater and terrestrial environments [1, 2, 3, 4]. Truncatelloidea has a rich fossil record, with the oldest fossil of this superfamily, *Triasamnicola pilsbryi* Yen & Reeside, 1946, dating back to the Late Triassic [5].

The species comprising this superfamily are all micro to small in size, typically ranging from 0.7 mm (Spirostyliferinidae Layton, Middelfart, Tatarnic & N. G. Wilson, 2019) to 15 mm (Bithyniidae Gray, 1857), posing challenges to taxonomic studies in the past. Most species of Truncatelloidea were assigned to Rissooidea Gray, 1847 solely based on shell morphology. However, with the help of the molecular phylogeny based on 16S and 28S markers [6], Truncatelloidea has been considered as a distinct monophyletic lineage from Rissooidea and given superfamily rank. Several subsequent

molecular studies have confirmed the validity of this superfamily [7, 8, 9]. A monotypic family within Truncatelloidea has been established for the marine microgastropod genus *Spirostyliferina* Bandel, 2006 [10]. A molecular phylogeny has revealed that family Caledoniellidae Rosewater, 1969 belongs to Truncatelloidea [11]. Two truncatelloidea families, Baicaliidae Fischer, 1885 and Erhaiidae. Davis & Kuo, 1985, have been considered as valid based on morphological and molecular study, and a fossil family Palaeobaicaliidae Sitnikova & Vinarski, 2023 † from the Late Cretaceous has been proposed [12]. A monotypic family, Litthabitellidae Falniowski & Hofman, 2023, has been erected for the freshwater truncatelloidean genus *Litthabitella* Boeters, 1970 [13].

The Oriental realm is one of the regions with relatively high biodiversity of Truncatelloidea, with a record of 18 families [1]. Several families of Truncatelloidea, such as Pomatiopsidae W. Stimpson, 1865 and Stenothyridae Tryon, 1866, exhibit remarkable diversity in the freshwater habitat of Mekong River basin in Thailand and Yunnan Plateau in China [14, 15, 16, 17, 18]. Numerous new taxa of Truncatelloidea recently have been described from Oriental realm, such as *Vietricula* Dang & Ho, 2010 from Vietnam [19], subterranean *Pseudoiglica* Gergo, 2018 from Laos [20], a new genus and species of Clenchiellidae D. W. Taylor, 1966 from Hongkong, China and Iriomote Island, Japan [21], and a new species of *Lacunopsis* Deshayes, 1876 from Fuxian Lake, Yunnan, China [22].

Fuxian Lake is a freshwater ancient lake located in central Yunnan, China. It is the deepest (155 m) and third largest (212 km^2) lake in Yunnan, and was formed due to tectonic activities in the Yunnan Plateau about 3.4 Ma [23]. Fuxian Lake harbours a high diversity of endemic fishes and freshwater snails, including 23 native species and subspecies of fish, of which 10 species are endemic [24], and 18 reported native species of freshwater gastropod, of which 6 species are endemic [22, 25]. Among the 6 endemic species of freshwater gastropod, 5 species belong to Pomatiopsidae. However, the true diversity of freshwater gastropod in Fuxian Lake is still poorly understood due to the lack of comprehensive and professional sampling.

In March of 2022, a batch of freshwater gastropod specimens was collected in Fuxian Lake. Among the specimens, a microgastropod species from a single locality caught our particular attention. The shell and operculum of this species are distinguished from those of any known species from Fuxian Lake. We conducted a detailed morphological, anatomical and molecular study of this species. These multiple sources of evidence support it as a new species of a new genus within an unknown family of Truncatelloidea. This paper presents the first fossil-calibrated phylogeny of Truncatelloidea covering most known families based on two mitochondrial (COI, 16S) and three nuclear loci (18S, 28S, H3), and describes a new truncatelloidean family , Squamapicidae fam. nov., only containing one new species, *Squamapex taiji* gen. et sp. nov. endemic to Fuxian Lake.

Material and methods

Materials

Living animals were collected on March 27, 2022 from Fuxian Lake. They were kept for observation for two to three weeks in an aquarium using treated tape water in Kunming, Yunnan, China. The

animals were later fixed as specimens using analytical grade pure ethanol. The type specimens of the new species are kept in the collection of Zoological Museum of China, Institute of Zoology, Chinese Academy of Sciences, Beijing (IZCAS) and Museum für Naturkunde, Berlin (ZMB). Six type specimens of *Lacunopsis yunnanensis* Liu *et al.*, 1980 kept in IZCAS and two specimens of *Lacunopsis harmandi* Poirier, 1881 kept in ZMB were used for comparison.

Examination of morphology

The shell height (H) and width (W) were measured with a calliper to a precision of 0.1 mm. The specimens were photographed in a consistent orientation using focus stacking methods with a Sony alpha 7R 4 camera. LAS X-software were used to do measurements and focus stacking. Radulae were extracted through dissection; radulae were cleaned by boiling in 1% NaOH solution for half an hour, and rinsed with distilled water. Radulae, operculum and protoconch were coated with gold before scanning electron microscopy with a Zeiss *EVO LS10* scanning electronic microscope. The body of snail were taken out from shell during dissection and photographed under a stereomicroscope (Leica MDG34). The terms used for describing the operculum are according to Zhang and von Rintelen [26].

DNA extraction and amplification

Genomic DNA was extracted from a whole animal using a mollusc-specific CTAB/chloroform extraction protocol [27]. Two mitochondrial loci (COI and 16S) and two nuclear loci (H3 and 28S) were targeted, and partial sequences were amplified through polymerase chain reaction (PCR) with the use of the following primer pairs: LCO1490, 5′-GGTCAACAAATCATAAAGATATTGG-3′ and COX-B7R, 5′-ACCACCAGCTGGATCAAAAA-3 [28] for COI; 16Sar-L, 5′-CGCCTGTTTATCAAAAACAT-3′ and 16Sbr-H, 5′-CCGGTCTGAACTCAGATCACGT-3′ [29], for 16S. PCR amplifications of COI and 16S were conducted in volumes of 25 µl under the following cycling conditions: initial denaturing step at 94 °C for 10 min, followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min (COI) or 52 °C for 30 s (16S), and 72 °C for 1 min, with a final extension step of 10 min at 72 °C. 28S and H3F were amplified by using primers and cycling conditions in Van Boexlaer *et al*. [30]. The purification and sequencing were conducted by Macrogen Europe, Amsterdam, Netherlands.

Phylogenetic analysis

Newly generated sequences for *Squamapex taiji* gen. et sp. nov. aligned with Truncatelloidea sequences obtained from Genbank (Supplementary table 1) for all four markers. Since we used data from multiple studies, we decided to build an extra alignment with 18S ribosomal subunit sequences to compensate for the gaps. We did not sequence our specimen for that marker, but we found that the added information contributed to convergence. The protein-coding markers were aligned by MUSCLE [31, 32] as implemented in MEGA [33] whereas ribosomal markers were manually aligned according to their secondary structure (Supplementary table 2). Regions of non-homology were removed from the alignments. We then used jModelTest 2.1 [34] to assess the best substitution model using the corrected Akaike Information Criterion (AICc).

All five alignments were then used as input in BEAST v. 2.7.4 [35]. We used the Standard template and all trees were linked while clocks and models were kept independent. We used the bModelTest [36] to estimate substitution models but used the results of jModelTest to choose the Model Set parameter. For the clock model, we used the Optimised Relaxed Clock package [37, 38]. We used Calibrated Yule for the tree model and set the birth rate prior to a lognormal distribution. For the time calibration, we used the fossil *Triasamnicola pilsbryi* Yen & Reeside 1946. We used the data from Kirby [5] to set the prior for this calibration point: offset = 201.0, M = 7.0, S = 0.9, mean set in real space. We used only this calibration point because family- /genus-level identification of gastropod fossils is difficult and we could not establish which fossils were crown or stem-group. This problem caused a conflict in the length of branches that made convergence in the Bayesian chain impossible. The BEAST analysis was run for 100 million generations sampling every 10 thousand. The resulting log was assessed in Tracer 1.7 [39] and, accordingly, a burn-in of 25% was used in TreeAnnotator. The run was repeated twice to assess replicability.

Results

Time-calibrated phylogeny

In this study, we present the first time-calibrated phylogeny of superfamily Truncatelloidea based on 194 sequences from 59 species, representing 30 of 33 known extant truncatelloidean families and one new family. This data set is the most comprehensive one for the family-level phylogeny of Truncatelloidea so far. We only employed the oldest and most reliable fossil calibration in taxonomy of this superfamily, *Triasamnicola pilsbryi* from the Chinle Formation of Late Triassic, to estimate divergence times.

Truncatelloidea is divided into two main clades, clade A and B. However, only clade B is well supported (pp=0.94). Clade A includes 10 families: Caecidae, Caledoniellidae, Calopiidae, Clenchiellidae, Elachisinidae, Iravadiidae, Spirostyliferinidae, Teinostomatidae, Tornidae, and Vitrinellidae. Clade B includes the rest 21 families: Amnicolidae, Anabathridae, Assimineidae, Baicaliidae, Bithyniidae, Bythinellidae, Cochliopidae, Emmericiidae, Erhaiidae, Falsicingulidae, Fontigentidae, Hydrobiidae, Hydrococcidae, Lithoglyphidae, Moitessieriidae, Pomatiopsidae, Squamapicidae fam. nov., Stenothyridae, Tateidae, Tomichiidae, and Truncatellidae.

The members of clade A are mostly found in the marine environment, or have a much higher diversity in marine/brackish environments compared to freshwater (viz. Clenchiellidae, Iravadiidae, Tornidae). In clade A, Caecidae is the sister group of Caledoniellidae (pp=0.99, node age=130.26 Ma); the clade of above two families is the sister group of Teinostomatidae (pp=1, node age=138.17Ma); the clade comprising above three families is the sister group of Elachisinidae (pp=0.97, node age=157.27 Ma); Calopiidae is the sister group of Clenchiellidae (pp=1, node age=86.35Ma); Vitrinellidae is the sister group of Spirostyliferinidae (pp=0.93, node age=155.71Ma). The other phylogenetic relationships within clade A are not quite reliable due to low support values. The diversifications of families in clade A mostly occurred during the Late Jurassic to the Early Cretaceous, except for the split of Calopiidae and Clenchiellidae in the late Cretaceous. The diversifications within two families of clade

A, viz. Caledoniellidae (pp=1, node age=46.45Ma) and Tornidae (pp=1, node age=42.93Ma), occurred in the Eocene.

Most members of clade B are found in freshwater environment, or have a much higher diversity in freshwater comparing in marine (viz. Cochliopidae, Hydrobiidae, Stenothyridae, Tateidae). In clade B, 18 families except for Tateidae, Cochliopidae and Hydrococcidae cluster together and form a well-supported subclade (pp=0.95, node age=123.25 Ma); 16 of the above 18 families except for Anabathridae and Hydrobiidae cluster together and form a well-supported subclade (pp=0.94, node age=113.67 Ma). The subclade of 16 families can be further divided into three well supported clades: (I) a clade only includes Squamapicidae fam. nov. and Stenothyridae, which are sister groups to each other (pp=1, node age=74.08 Ma); (II) a clade includes Assimineidae, Falsicingulidae, Pomatiopsidae, Tomichiidae and Truncatellidae (pp=0.99, node age=94.00 Ma); within this clade, Falsicingulidae, Pomatiopsidae and Tomichiidae further cluster together (pp=1, node age=62.04 Ma); The only three truncatelloidean families which evolve terrestrial species (Assimineidae, Pomatiopsidae and Truncatellidae,) are all in this clade. (III) a clade includes Amnicolidae, Baicaliidae, Bithyniidae, Bythinellidae, Emmericiidae, Erhaiidae, Fontigentidae, Lithoglyphidae and Moitessieriidae (pp=0.99, node age=94.03 Ma); a sub-clade including Amnicolidae, Baicaliidae and Erhaiidae (pp=1, node age=58.95 Ma) is the sister group of Bithyniidae (pp=0.98, node age=77.78 Ma); Bythinellidae, Emmericiidae, Fontigentidae and Lithoglyphidae cluster together and form another sub-clade (pp=0.93, node age=74.05). The initial diversification of clade B started in the Early Cretaceous (node age=140.14). The diversification of three clades all happened in the Late Cretaceous. The diversifications within ten families of clade B happened in the Paleogene, and five of these ten families occurred in the Eocene (Erhaiidae: pp=1, node age=37.16 Ma; Bithyniidae: pp=1, node age=36.12 Ma; Moitessieriidae: pp=1, node age=50.97 Ma; Assimineidae: pp=1, node age=57.15 Ma; Pomatiopsidae: pp=1, node age=39.72 Ma; Tomichiidae: pp=1, node age=42.04 Ma; Falsicingulidae: pp=1, node age=26.87 Ma;Stenothyridae: pp=1, node age=40.65 Ma; Hydrobiidae: pp=1, node age=65.90 Ma).

Systematics

Superfamily **Truncatelloidea Gray, 1840**

Family **Squamapicidae fam. nov.**

Type genus: *Squamapex* Zhang gen. nov.

Diagnosis

Shell micro, number of whorls low; two-stage protoconch, first half protoconch whorl with weak sparse veins, the rest protoconch whorl strongly ornamented, covered with many lines of regularly distributed large square-scale-shaped pits, teleoconch without complex microsculpture; operculum kidney-shaped, inner opercular region hourglass-shaped, with two different projections on the upper and lower part of inner opercular region; central teeth of radula trident-head-shaped, with three sharp triangle cusps and a pair of long basal denticles.

Remarks

Squamapicidae is a monotypic family of Truncatelloidea, containing a monotypic genus *Squamapex* gen. nov. and one species *Squamapex taiji* sp. nov.. The characters of radula and head-foot support that Squamapicidae belongs to Truncatelloidea. However, this family is distinct from any other known families within Truncatelloidea due to its two stage square-scale-shaped pitted protoconch and operculum with two projections on the hourglass-shaped inner opercular region. Marine snail Spirostyliferinidae is another truncatelloidean family with complex microsculpture on the protoconch. Some species of Moitessieriidae Bourguignat, 1864, such as *Moitessieria boetersi* Girardi, 2015, have strongly ornamented protoconch and teleoconch. However, both of them are quite distinct from Squamapicidae.

Distribution

This family is only known from one freshwater ancient lake, Fuxian Lake, in Yunnan, China.

***Squamapex* gen. nov.**

Type species: *Squamapex taiji* Zhang sp. nov.

Diagnosis

Same as family Squamapicidae.

Etymology

Squamapex is derived from 'squama' (Latin for scale) and 'apex' (Latin for top), in reference to the scale-like pits on the protoconch. The recommended Chinese name is "鳞顶螺".

***Squamapex taiji* sp. nov.**

Material examined

Holotype: IZCAS, collected on March 27, 2022 by Le-Jia Zhang from a small rock about 0.1 m deep in the west coast of Lake Fuxian near Jianshan Village, Chengjiang City, Yunnan, China.

Paratypes: IZCAS, 18 individuals, from the same rock inhabited by the holotype, same collecting data as the holotype. ZMB, 2 individuals, from the same rock inhabited by the holotype, same collecting data as the holotype.

Additional material: 1 subfossil collected from the east coast of Fuxian Lake, near Haikou Village, private collection of Hong-Quan Xiang.

Description

Shell minute, width 1.56 ± 0.11 (1.22~1.76) mm, height 1.04 ± 0.07 (0.82~1.12) mm, dextral, depressed cap-shaped, thin but solid, orange to reddish brown in colour; mostly with three whorls, including one and a half protoconch whorls with dense pits, one and a half teleoconch whorls with fine

growth lines, spiral whorls short and depressed, body whorl large and wide, shell base flat; aperture kidney-shaped; outer lip thin but solid, upper, central and lower part extended, forming a wide M-shaped margin; inner lip extremely extended, forming a flat transparent glassy semi-circular structure, covering umbilicus completely and nearly three-fourth part of shell base.

Protoconch broad, one and a half whorls, low dome-shaped, first half whorl with weak sparse veins, the rest one whorl with 14 to 16 rows of regularly distributed large square-scale-shaped pits exposed, all square-scale-shaped pits in similar size.

Operculum (n=4) corneous, kidney-shaped, thin and fragile, transparent yellow in colour; subcentral paucispiral nucleus visible on exterior surface, close to inner margin and opercular base, on the upper and lower part each with a shallow apophysis; interior surface with a long hourglass-shaped inner opercular region, on the central of upper and lower part of inner opercular region each with a projection, oblong upper projection obviously longer than square lower projection, the lower end of upper projection and upper end of lower projection both pointing to the central of inner margin; enamel region flat and smooth, inner enamel region narrow and thickened; marginal region very thin and fragile, filling the concave region of central inner margin.

Radula (n=2) taenioglossate (2+1+1+1+2); central teeth trident-head-shaped, with two deep lower concave edge, upper edge with three sharp triangle cusps-one smaller cusp on either side of big and long median cusp, with one long large basal denticle on the central part of either lower concave edge; lateral teeth with two to three cusps on inner side and six cusps on outer sides; inner marginal teeth with 14-19 cusps, outer marginal teeth with 10-14 cusps.

Head-foot of ethanol-preserved specimens (n=3) transparent pale grey; a pair of eyebrow-shaped darker pattern on the base of each tentacle, behind each eye, another pair of eyebrow-shaped darker pattern on the base of either side of snout, under each eye; foot small, anterior part expanded laterally and anterior edge indented.

Morphological comparison

Squamapex taiji has a depressed cap-shaped shell with an extremely extended inner lip that covers the base. This character is somewhat similar to the shell of *Lacunopsis harmndi* Poirier, 1881 from NE Thailand and *Lacunopsis yuxiensis* Liu, Wang & Zhang, 1980 also from Fuxian Lake, both belonging to family Pomatiopsidae Stimpson, 1865. However, both species are much larger in size (width of *L. harmndi* about 7 mm; width of *L. yuxiensis* about 3 mm), and have a simple outer lip, a smooth protoconch and a simple oval operculum; the radula, especial the central teeth and more whorls (4.5) of *L. yuxiensis* is distinct from those of *Squamapex taiji* as well.

Ecology and distribution

Densely inhabiting rocks on strongly wave-exposed the lake shore, close to the river mouth, not coexisting with any other mollusc; living animals are only known from the type locality near Jianshan

Village in Fuxian Lake, Yunnan, China; one subfossil is recorded from a locality near Haikou Village in Fuxian Lake. The living animals were very active and moved fast in aquarium.

Etymology

The overall shape of inner lip and aperture is similar to the Chinese yin-yang symbol, Taiji. Formed as a noun in apposition. The recommended Chinese name is “太极鳞顶螺”.

Discussion

Our integrative study strongly supports that the abnormal freshwater miscrogastropod from Fuxian Lake is a new species of a new genus within a new family of Truncatelloidea. The time-calibrated phylogeny based on five loci confirms that Squmapicidae belongs to Truncatelloidea and is a quite distinct and ancient lineage. The split of Squmapicidae and its sister clade Stenothyridae occurred in the Late Cretaceous, which is much earlier than the oldest reliable record of Stenothyridae from the Middle Eocene [40]. Morphologically, the micro size, head-foot and radular characters support it as a member of Truncatelloidea. However, the protoconch with square-scale-shaped pits, operculum with two thick projections on the hourglass-shaped inner opercular region and unique central tooth of radula of this species make it hardly assigned to any known family. Most families of Truncatelloidea only have a smooth or weakly ornamented protoconch and a thin flat operculum [10]. Stenothyridae has a round operculum with two equal-sized parallel thin projections [8], which is the only morphological clue indicating its close relationship with Squmapicidae.

Squamapex taiji has one of the most unique protoconchs among freshwater gastropods. The first half protoconch whorl only has weak veins, but remining protoconch whorl (one whorl in total) is strongly ornamented with square-scale-shaped pits. The teleoconch whorls have a completely different surface microstructure. Such a two-stage ornamented protoconch with distinct microstructure from the teleoconch is quite rare for true freshwater gastropods (if not the only case), but relatively common for many marine or migratory gastropods with veliger larvae [41]. As for these gastropods, the first stage protoconch is the embryonic shell before hatching, and the second stage protoconch is the veliger larval shell during the planktonic stage. The microsculpture of the protoconch is supposed to prevent or retard mechanical damage in veliger larva, and should adaptively protect them against predators [42]. A veliger larva has been observed in marine species of Truncatelloidea, such as *Peringia ulvae* (Pennant, 1777), which has a two-stage protoconch with a smooth first-stage shell and a second-stage shell with simple weak spiral lines [43]. As far as we know, a veliger larvae has never been discovered in freshwater Truncatelloidea species or any other gastropods with a life history completely in freshwater. The protoconch of *Squamapex taiji* is a very remarkable case within freshwater gastropods, probably indicating a larva dwelling in a different habitat in comparison to the benthic adult. However, we can only test this conjecture based on a more extensive ecological study of the living animals.

Our molecular phylogenetic analyses provide the most complete phylogenetic framework at family level of Truncatelloidea so far. The phylogenetic position and relationships of not only the new family Squmapicidae, but also many other truncatelloidean families, are much clearer and more robust in our

phylogeny. The two major clades recovered here are consistent with the results of previous phylogenies involving Truncatelloidea [6, 7, 10, 11]. The topology of two highly supported clades I &II of the clade B is quite similar to the result of Layton et al. [10], even though Baicaliidae, Fontigentidae, Lithoglyphidae and Tomichiidae were missing in their analysis. The close relationship and phylogenetic topology of three families of clade A, Caecidae, Caledoniellidae and Teinostomatidae, is identical to the result from Goto et al. [11]. A highly supported sub-clade of clade II including Falsicingulidae, Pomatiopsidae, Tomichiidae recovered here is consistent with the result of Salvador [44]. Our phylogeny supported a former study only based on COI showing that Fontigentidae is a valid family distinct from Emmericiidae, and is the sister group of Bythinellidae [45]. Additionally, our phylogeny confirms that two former subfamilies from Amnicolidae, viz. Erhaiidae and Baicaliidae, should both be elevated to family level proposed in a former study [12].

The fossil record is widely regarded as providing the most reliable information for the calibration of phylogeny. Nevertheless, the accuracy of taxonomic identification, dating and the phylogenetic placement of fossils can significantly influence molecular clock analyses [46]. The shells of mollusks can exhibit a large degree of similarity due to convergent evolution, e.g., between three genera of Viviparidae endemic to lakes of Yunnan, China [47], or between *Spirostyliferina* of Spirostyliferinidae within Truncatelloidea and *Stilifer* of Eulimidae within Vanikoroidea [10]. The fossil calibration used here, *Triasamnicola pilsbryi* from Late Triassic, can be unambiguously identified as the oldest known member of Truncatelloidea based on its shell characters, small size and freshwater habitat [48, 49]. However, the previous assignment of this oldest species to Amnicolidae is obviously wrong according to our phylogeny, since Amnicolidae is a relatively young lineage instead of a basal clade of Truncatelloidea. It is more reasonable to place this fossil species into an undescribed extinct family of Late Triassic instead of a modern family.

Despite the use of only one fossil calibration in our analysis, the estimated divergence time is found to be in perfect agreement with the age of a lot of fossil records. There are a large number and high diversity of fossil records of Stenothyridae, such as the extinct genera *Stenothyroides* only found in Western Europe from Middle Eocene to Early Miocene [40, 50, 51] and a dozen extinct *Stenothyra* species discovered in the sediments of Paleolake Nanning of southern China from Oligocene [52]. The time of these fossil records are consistent with the estimated divergence time within Stenothyridae (40.64 Ma and 28.85 Ma). There are abundant fossil records of the extinct freshwater family Palaeobaicaliidae Sitnikova & Vinarski, 2023 † from Late Cretaceous to Paleogene in Northern Asia and northern China, which is morphologically similar to Baicaliidae from Northern Asia as well (Lake Baikal and lower Angara River) [12]. Sitnikova et al. [12] estimated that the divergence of the clade including Amnicolidae, Erhaiidae and Baicaliidae happened in the Middle Miocene based on an analysis using the divergence rate of COI. However, our analysis based on mitochondrial & nuclear loci and a fossil calibration indicates that the divergence time of this clade should be dated to the Late Paleocene (58.95 Ma), which is roughly consistent with the age of Palaeobaicaliidae. The observed consistency across different groups support that the placement of the fossil used for calibration and the resulting age estimates are reasonable.

The sister group of Squamapicidae, Stenothyridae, displays an obvious Tethys Ocean origin based on its partial brackish/marine habitats and Eocene-modern distribution in Asia-Australia [8, 53] and Eocene-Miocene distribution in Europe [40]. The Persian Gulf and the surrounding freshwater environment form the westernmost limit of the distribution of extant Stenothyridae [8, 54]. The extinction time of Stenothyridae in the Mediterranean region after the Middle Miocene is consistent with the closing time of the Tethys Seaway (of which the Persian Gulf is a relic) connecting the Mediterranean and the Indian Ocean [55]. We have not found any fossil morphologically similar to Squamapicidae. Therefore, there is no direct evidence of a Cretaceous Tethys origin of this family. However, the Tethys Ocean existed and extended to NW Yunnan of Himalayan region, until the Late Eocene [56]. Coupled with the evidence of the unique protoconch and the age estimate derived from our time-calibrated phylogeny, there is a high possibility that Squamapicidae and Stenothyridae evolved from a marine common ancestor in the Tethys Ocean of the Late Cretaceous. Unlike Stenothyridae with high species diversity successfully and widely inhabiting coastal regions, the monospecific family Squamapicidae is the only descendant of its lineage. It had to adapt to a true freshwater environment in Yunnan after the complete closure of the Tethys Ocean in the Himalayan region.

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Data accessibility

The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions

L. J. Z. collected specimens, took photos, did measurements, carried out laboratory work, participated in the study design, did the data analysis, drafted and revised the manuscript; S. C. B. participated in the study design, did the data analysis, drafted and revised the manuscript; K. M. took photos and contributed in species identification. T. v. R. participated in data analysis and revised the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

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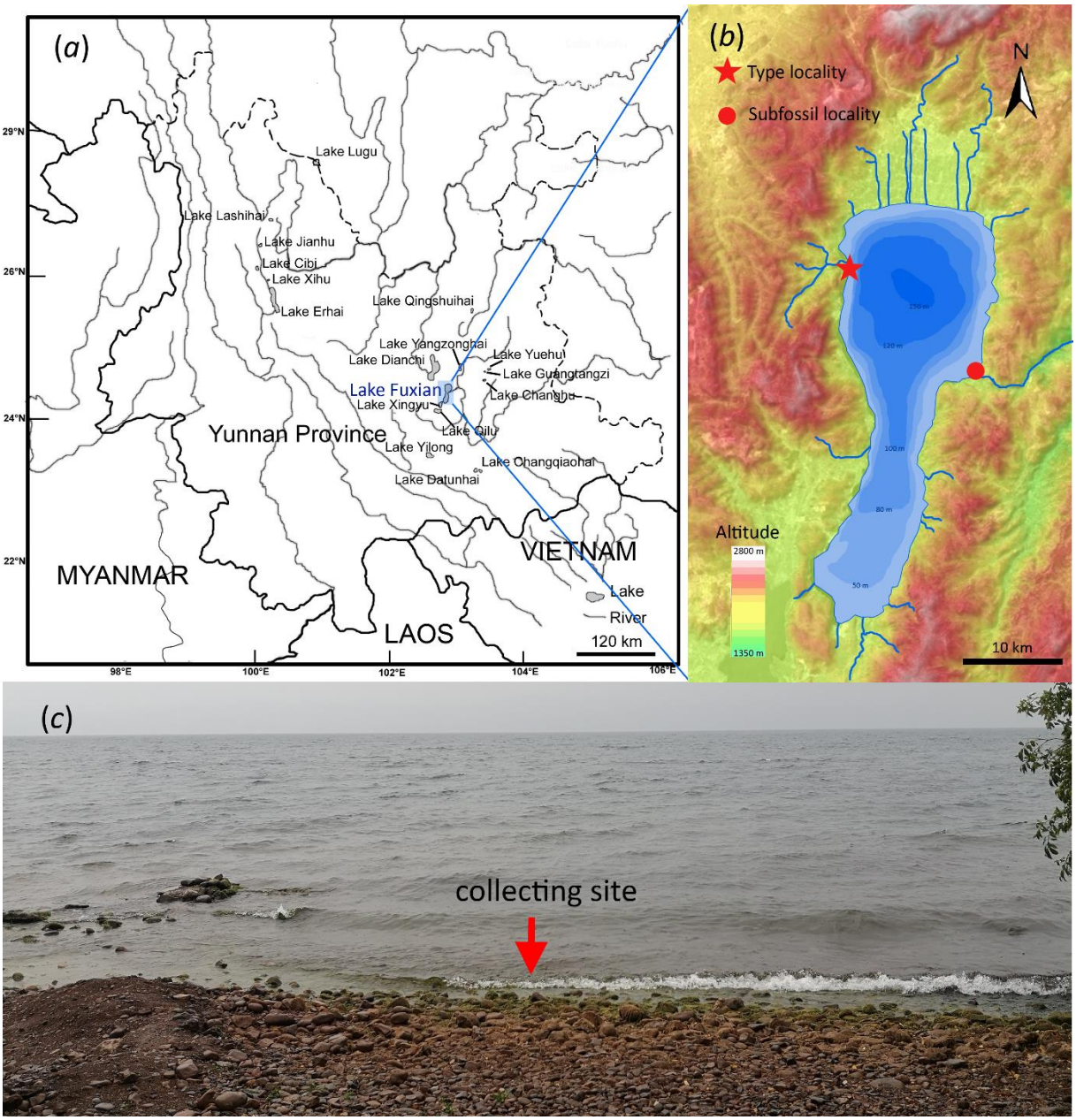
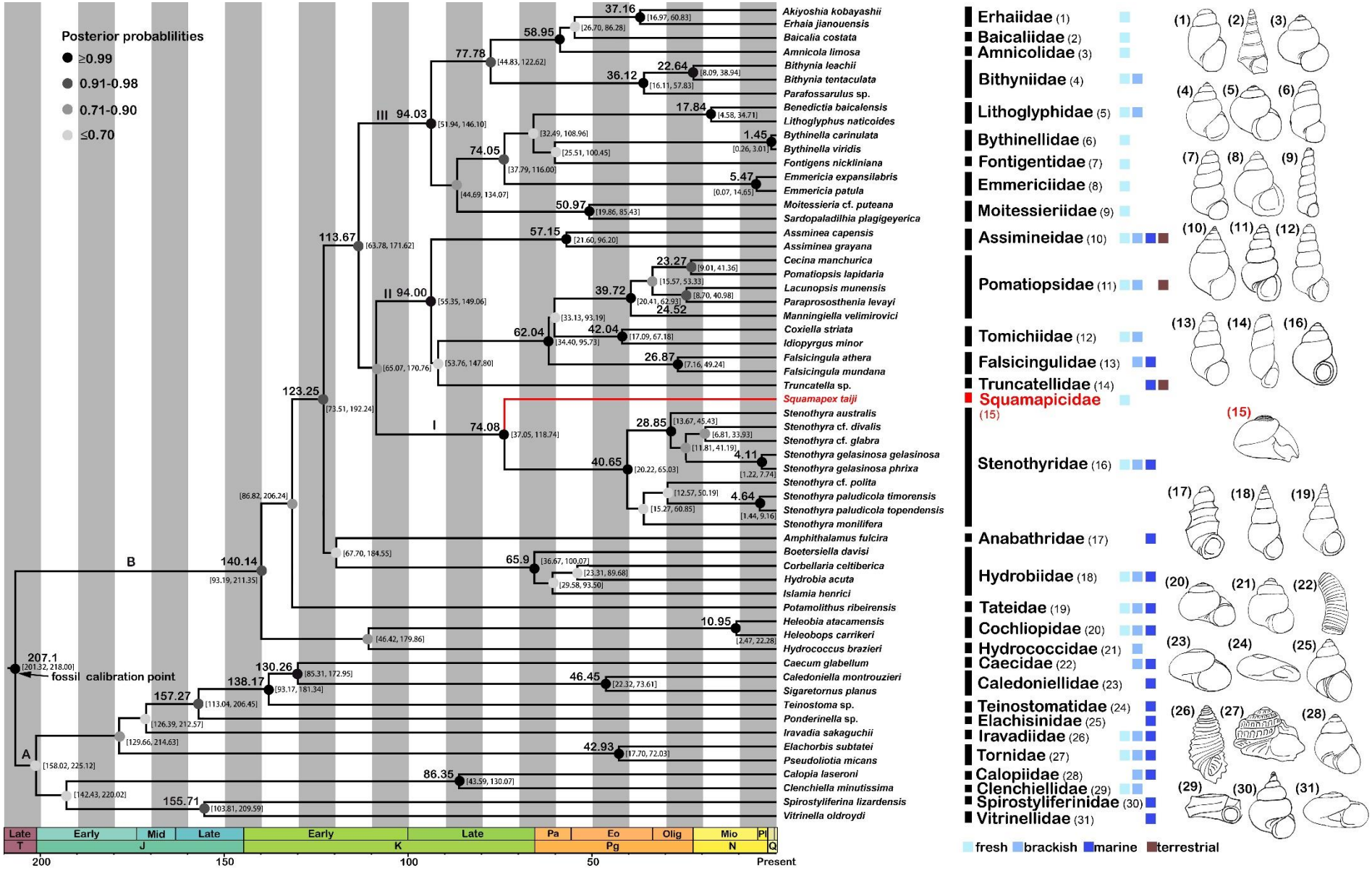


Figure 1. Collecting sites and habitats of *Squamapex taiji* gen. et sp. nov., (*a*), map of Yunnan Province showing the location of Fuxian Lake, modified from [47]; (*b*), collecting sites in Fuxian Lake, modified based on the resources from https://topographic-map.com; (*c*), environment of the type locality.


Posterior probablilities
≥0.99
0.91-0.98
0.71-0.90
≤0.70
Akiyoshia kobayashii
Erhaia jianouensis
Baicalia costata
Amnicola limosa
Bithynia leachii
Bithynia tentaculata
Parafossarulus sp.
Benedictia baicalensis
Lithoglyphus naticoides
Bythinella carinulata
Bythinella viridis
Fontigens nickliniana
Emmericia expansilabris
Emmericia patula
Moitessieria cf. puteana
Sardopaladilhia plagigeyerica
Assminea capensis
Assiminea grayana
Cecina manchurica
Pomatiopsis lapidaria
Lacunopsis munensis
Paraprososthenia levayi
Manningiella velimirovici
Coxiella striata
Idiopygus minor
Falsicingula athera
Falsicingula mundana
Truncatella sp.
Squamapex taiji
Stenothyra australis
Stenothyra cf. divalis
Stenothyra cf. glabra
Stenothyra gelasinosa gelasinosa
Stenothyra gelasinosa phrixa
Stenothyra cf. polita
Stenothyra paludicola timorensis
Stenothyra paludicola topendensis
Stenothyra monilifera
Amphithalamus fulcira
Boetersiella davisi
Corbellaria celtiberica
Hydrobia acuta
Islamia henrici
Potamolithus ribeirensis
Heleobia atacamensis
Heleobops carrikeri
Hydrococcus brazieri
Caecum glabellum
Caledoniella montrouzieri
Sigaretornus planus
Teinostoma sp.
Ponderinella sp.
Iravadia sakaguchii
Elachorbis subtatei
Pseudoliotia micans
Calopia laseroni
Clenchiella minutissima
Spirostyliferina lizardensis
Vitrinella oldroydi
fossil calibration point
Late
Early
Mid
Late
Early
Late
Pa
Eo
Olig
Mio
Pl
T
J
K
Pg
N
Q
200
150
100
50
Present

Erhaiidae (1)
Baicaliidae (2)
Amnicolidae (3)
Bithyniidae (4)
Lithoglyphidae (5)
Bythinellidae (6)
Fontigentidae (7)
Emmericiidae (8)
Moitessieriidae (9)
Assimineidae (10)
Pomatiopsidae (11)
Tomichiidae (12)
Falsicingulidae (13)
Truncatellidae (14)
Squamapicidae (15)
Stenothyridae (16)
Anabathridae (17)
Hydrobiidae (18)
Tateidae (19)
Cochliopidae (20)
Hydrococcidae (21)
Caecidae (22)
Caledoniellidae (23)
Teinostomatidae (24)
Elachisinidae (25)
Iravadiidae (26)
Tornidae (27)
Calopiidae (28)
Clenchiellidae (29)
Spirostyliferinidae (30)
Vitrinellidae (31)
fresh
brackish
marine
terrestrial

Figure 2. BEAST MCC tree of Truncatelloidea based on partial sequences of COI, 16S, 18S, 28S and H3 including posterior probabilities (pp), node ages (only when pp ≥0.91), and 95% HPD.

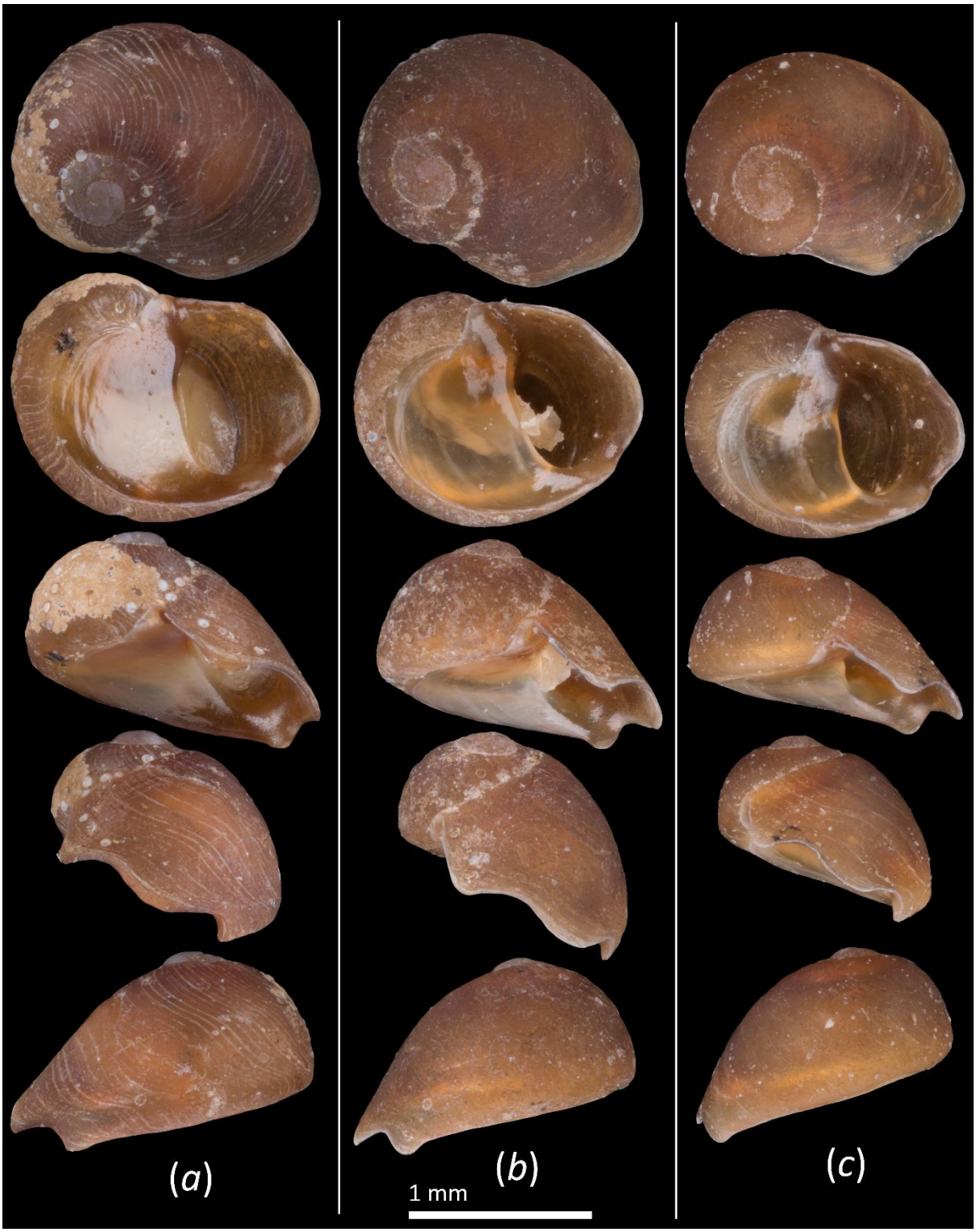


Figure 3. Shell of *Squamapex taiji* gen. et sp. nov.. (*a*) Holotype, IZCAS. (*b*) to (*c*) Paratypes, IZCAS. All shells share the same scale bar.

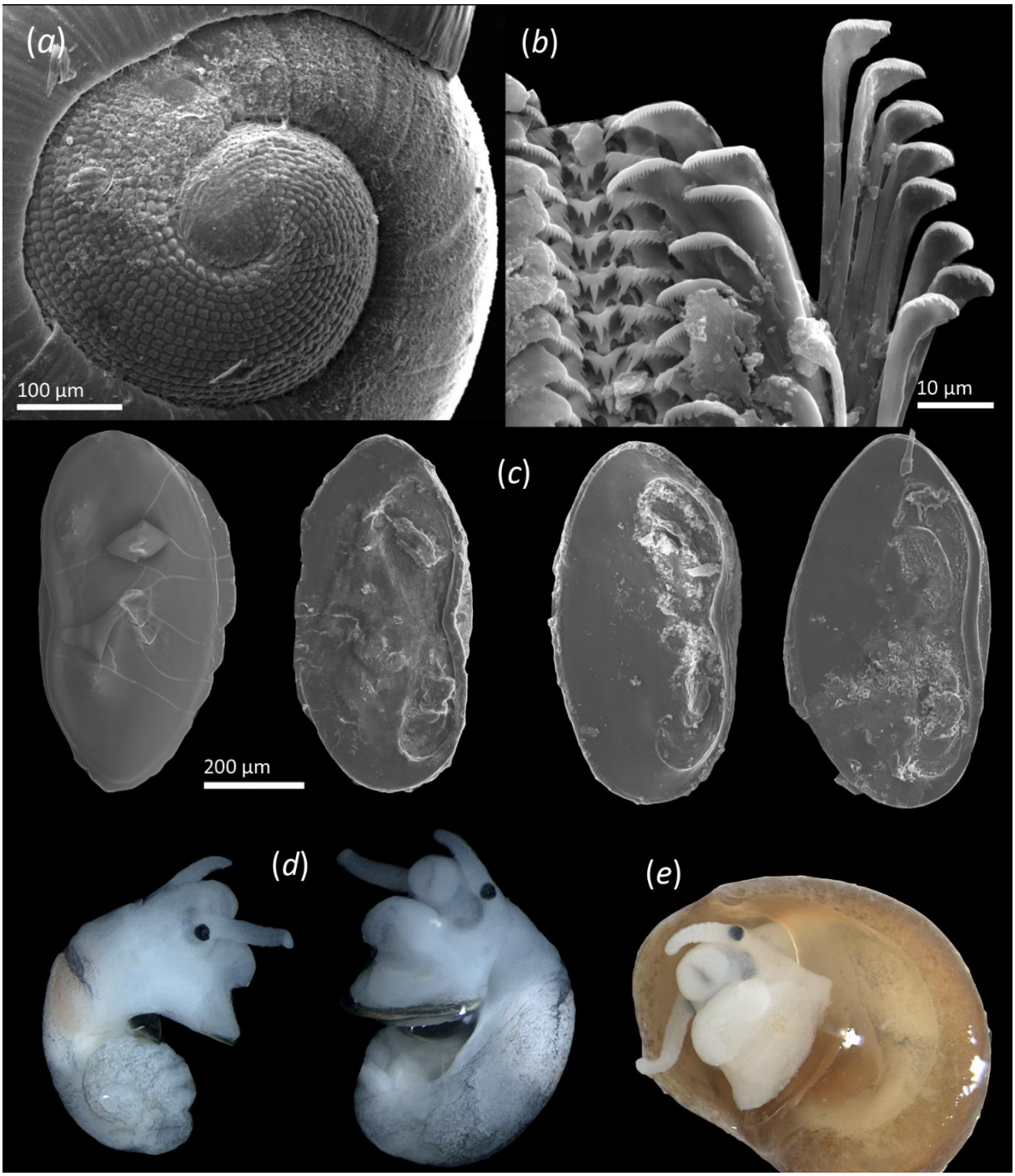


Figure 4. Morphological details of *Squamapex taiji* gen. et sp. nov.. (*a*) SEM photo of the protoconch. (*b*) SEM photo of the radula. (*c*) SEM photo of the operculum. (*d*) The soft body of a specimen kept in ethanol showing the head-foot part, IZCAS. (*e*). A specimen with shell kept in ethanol, IZCAS.

Supplementary table 1: Sequences used in this study

	COI	**16S**	**18S**	**28S**	**H3**
Akiyoshia kobayashii	AB611823	AB611822	AB611820	AB611821	
Amnicola limosa	KM612163	AF212903	AF212916		
Amphithalamus fulcira	AB930477	AB930417	AB930390	AB930345	AB930444
Assiminea capensis	KC439797	KC109939		KC109991	
Assiminea grayana	EF667310	EF667338	HQ623162		
Baicalia costata	JX970599	JX970525	JX970558		
Benedictia baicalensis	HQ623171	AF445339	HQ623163		
Bithynia leachii	MT410857	MT410857			
Bithynia tentaculata	MK308073	AF445344	AF367675	FJ160289	
Boetersiella davisi	MH350198	MH347965	MH348096	MH348035	
Bythinella carinulata	FJ029099	FJ028884		FJ028896	
Bythinella viridis	FJ029102	KP765289		KP765254	
Caecum glabellum	AB930481	LC598168	AB930393	AB930352	AB930447
Caledoniella montrouzieri	LC598182	LC598170	LC598106	LC598122	LC598136
Calopia laseroni	LN831293	LN831288		KC439959	
Cecina manchurica	AB611747	AB611746	AB611740	AB611741	
Clenchiella minutissima	HG973061	KC109946	LC598111	KC109998	
Corbellaria celtiberica	MH350207	MH347974	MH348099	MH348044	
Coxiella striata	ON426794	KC109948	JX970593	KC110000	
Elachorbis subtatei	KC439807	KC109953		KC110005	
Emmericia expansilabris	KC810060	EU573985	EU573995		
Emmericia patula		JX970536	JX970572		
Erhaia jianouensis	AF367652	AF212894	AF212907		
Falsicingula athera	HQ623172	HQ623155	HQ623164		
Falsicingula mundana	KC439798	KC109957	AB930398	AB930366	AB930452
Fontigens nickliniana	MT425027		JX970573		
Heleobia atacamensis	OM490401	OM490268			OM489928
Heleobops carrikeri	JQ973018	AF212902	AF212915		
Hydrobia acuta	KC439801	KC109959	AF367680	KC110011	
Hydrococcus brazieri		KC109960	JX970579	KC110012	
Idiopyrgus minor	ON720330	ON720564		ON720562	
Iravadia sakaguchii	AB930473	AB930415	AB930387	AB930339	
Islamia henrici	MH350223	MH347990	MH348104	MH348060	
Lacunopsis munensis	KC832705	KC832726			
Lithoglyphus naticoides	AF354770	FJ160287	AF367674	FJ160290	
Manningiella velimirovici	KC832695	KC832716			
Moitessieria cf *puteana*	AF367635	EU573992	AF367665		
Parafossarulus sp.	HQ623174	HQ623158	HQ623166	MT497283	
Paraprososthenia levayi	KC832687	KC832708			
Pomatiopsis lapidaria	AF354774	AY676118	AF367666		
Ponderinella sp.			LC598113	LC598128	LC598143
Potamolithus ribeirensis	JX970618	JX970549	JX970591		
Pseudoliotia micans	KC439806	KC109971		KC110023	
Sardopaladilhia plagigeyerica	HQ623176	HQ623160	HQ623168		
Sigaretornus planus	LC598185		LC598109	LC598125	LC598139
Spirostyliferina lizardensis		MN119719	MN119726	MN119727	MN119715

Squamapex taiji	XXXXXX*	XXXXXX*		XXXXXX*	XXXXXX*
Stenothyra australis	KC439701	KC439823		KC439916	
Stenothyra cf. *divalis*	KC439745	KC439867		KC439930	
Stenothyra cf. *glabra*	MN548735	MN548735		MT090057	
Stenothyra cf. *polita*	KC439738	KC439860		KC439926	
Stenothyra gelasinosa gelasinosa	KC439712	KC439834		KC439919	
Stenothyra gelasinosa phrixa	KC439717	KC439839		KC439920	
Stenothyra monilifera	KC439737	KC439859		KC439925	
Stenothyra paludicola timorensis	KC439734	KC439856		KC439923	
Stenothyra paludicola topendensis	KC439731	KC439853		KC439922	
Teinostoma sp.	LC598189	LC598177	LC598117	LC598131	LC598148
Truncatella sp.	AB930482	AB930420		AB930353	AB930448
Vitrinella oldroydi		LC598179	LC598119	LC598133	LC598150

* - Sequences generated by this study

Supplementary table 2: Reference information for ribosomal secondary structure

Marker	**Species**	**Genbank accession no.**	**Reference for secondary structure**
16S	*Omalonyx unguis*	MT449229	doi:10.1371/journal.pone.0253724
18S	*Littorina littorea*	DQ093437	https://rnacentral.org/rna/URS000000D4EC/31216
28S	*Tonna allium*	LC646184	https://rnacentral.org/rna/URS00025AFCAD/2483503

4 DISCUSSION AND CONCLUSION

4.1 Taxonomy and systematics of Viviparidae from China

In the last two decades there has been significant progress in the taxonomic, systematic and biogeographic study of Viviparidae, especially in the groups from Africa (Sengupta *et al*., 2009; Schultheiß *et al*., 2014), China (Zhang *et al*., 2015; Van Bocxlaer *et al*., 2018), Japan (Hirano *et al*., 2015; Hirano *et al*., 2019a) and Southeast Asia (Stelbrink *et al*., 2019; Stelbrink *et al*., 2020). The present thesis is the first comprehensive work about the taxonomy and systematics of family Viviparidae from all over China. Two new genera and 10 new species (nine extant species & one fossil species) of Viviparidae were described in the present thesis. China now harbours nearly half of genera (14/31, 5 endemic) and one third of extant species (56/134-159, 42 endemic) of Viviparidae, displaying a much higher diversity than any other region of the world. A list of extant species of Viviparidae from China is given at the end of this section (Table 1), based on the present thesis, Hsü (1935), Yen (1943), Liu *et al*. (1995), Chen (2011), Zhang *et al*. (2015) and Hirano *et al*. (2019a).

Hirano *et al*., (2019b) considered that a species-level molecular phylogeny solely based on a single mitochondrial marker, such as COI, is problematic in Viviparidae. However, in this thesis, COI as a single marker performed quite well in the systematic study of Viviparidae, both at species and genus level. Within the two new viviparid genera *Bakyietaia* and *Dalipaludina*, a high degree of congruence between morphology-based species delimitations and species level units inferred from the COI phylogeny was found. The monophyly of most morpho-species (18/19) studied herein was supported by COI-based tree reconstructions. In addition, the two COI trees reconstructed for the *Bakyietaia* and *Dalipaludina* studies (in chapter 3 & 5) are both broadly consistent with the genus-level topology for the subfamily Bellamyinae in Stelbrink *et al*. (2020) based on three mitochondrial and nuclear genes; *Bakyietaia* falls into clade A, and *Dalipaludina* falls into clade B; the two clades of Bellamyinae were proposed by Stelbrink *et al*. (2020). As for the species with a high shell polymorphism , such as *Dalipaludina oxytropoides* and *Bakyietaia wilhelmi*, the results derived from COI were also in line with those from detailed morphological studies based on morphometric methods and radula structure (in chapter 5).

These results suggest that COI performs well in the taxonomic and systematic study of Viviparidae, being a simple but sufficient marker to establish baseline taxonomic hypotheses. However, the high plasticity of Viviparidae's shell has led to an overestimation of species diversity in the former studies. Many described "species" are probably only morphotypes or ecotypes. I consider this to be one of the main reasons causing the former problematic species-level phylogeny. Ye *et al*. (2021) have confirmed that seven morpho-species of *Sinotaia* should be synonymized with *Sinotaia quadrata*. Another example is the case of *Heterogen longispira* (Smith, 1886) endemic to Biwa Lake, *Heterogen* sp. endemic to Chikubu Island of Biwa Lake and the widespread *Heterogen japonica* (Martens, 1861); the three species can be easily distinguished based on their shell morphology, especially by characters of

the juveniles; however, they can hardly be differentiated either in a phylogeny based on COI and 16S (Hirano *et al*., 2015), a phylogeny based on five mitochondrial and nuclear genes (Hirano *et al*., 2019a), or even using genome-wide SNPs (Hirano *et al*., 2019a). All these results suggests the possibility that *Heterogen longispira* and *Heterogen* sp. rather represent lake ecotypes instead of species, and *Heterogen longispira* should be a synonym of *Heterogen japonica*.

In conclusion, the present thesis confirms that a comprehensive integrative study combining morphology and even a single DNA barcoding marker can provide a relatively robust taxonomical and systematic framework for Viviparidae at a lower taxonomic level (genera and species). In the future, genomic studies of Viviparidae, for example using exon capture, can provide a more robust phylogeny above genus-level and also help to clarify species boundaries in cases of recent divergence, such as likely encountered in *Bakyietaia*.

Tabel 1. List of extant species of Viviparidae from China.

Genus	Species	Distribution in China (Province)
Amuropaludina Moskvicheva, 1979	*Amuropaludina praerosa* (Gerstfeldt, 1859)	Jilin, Heilongjiang
Anularya Zhang & Chen, 2015*	*Anularya bicostata* (Tchang & Tsi, 1949)*	Yunnan
	Anularya mansuyi (Dautzenberg & Fischer, 1906)*	Yunnan
Bakyietaia gen. nov.	*Bakyietaia avisvenatoris* sp. nov.*	Guangdong, Guangxi
	Bakyietaia boettgeri (Heude, 1890)	Hainan, Guangxi
	Bakyietaia chenghuang sp. nov.*	Guangxi
	Bakyietaia duchieri (Fischer, 1898)	Guangxi
	Bakyietaia fontinalis sp. nov.*	Hainan
	Bakyietaia guangdungensis (Kobelt, 1906)*	Guangdong, Guangxi
	Bakyietaia jingweiae sp. nov.	Guangxi, Hainan
	Bakyietaia liangzhuorum sp. nov.*	Guangdong, Guangxi
	Bakyietaia liusanjieae sp. nov.*	Guangxi
	Bakyietaia luikongi sp. nov.*	Guangdong
	Bakyietaia luuemxlong sp. nov.*	Hainan
	Bakyietaia mutica (Kobelt, 1909)*	Hainan
	Bakyietaia naiadica sp. nov.*	Hainan
	Bakyietaia paradoxa sp. nov.*	Guangxi
	Bakyietaia polyzonata (Frauenfeld, 1862)	Guangxi
	Bakyietaia subcostata (Gray, 1833)*	Guangdong
	Bakyietaia wilhelmi (Yen, 1939)	Guangdong, Guangxi, Hainan, Yunnan
Cipangopaludina Hannibal, 1912	*Cipangopaludina aubryana* (Heude, 1890)*	Sichuan, Hunan, Guangdong, Guizhou, Yunnan
	Cipangopaludina catayensis (Heude, 1890)*	Jilin, Shanxi, Hebei, Shandong, Anhui, Jiangsu, Zhejiang, Hunan, Sichuan, Guizhou, Yunnan
	Cipangopaludina chinensis (J. E. Gray, 1833)	All provinces except for Tibet
	Cipangopaludina haasi Prashad, 1928*	Sichuan, Yunnan
	Cipangopaludina hainanensis (Kobelt, 1909)*	Hainan
	Cipangopaludina hehuensis Lu, Fang & Du, 2016*	Zhejiang
	Cipangopaludina latissima (Dautzenberg & Fischer, 1906)*	Yunnan

	Cipangopaludina lecythoides (Benson, 1842)*	Zhejiang
	Cipangopaludina menglaensis Zhang, Liu & Wang, 1981*	Yunnan
	Cipangopaludina miyagii Kuroda, 1941*	Taiwan
	Cipangopaludina yunnanensis Zhang, Liu & Wang, 1981*	Yunnan
Dalipaludina Zhang, 2023*	*Dalipaludina delavayana* (Heude, 1889)*	Yunnan
	Dalipaludina occidentalis (Annandale, 1924)*	Yunnan
	Dalipaludina oxytropoides (Heude, 1889)*	Yunnan
	Dalipaludina pyramidella (Du, Yang & Chen, 2011)*	Yunnan
Filopaludina Habe, 1964	*Filopaludina doliaris* (A. Gould, 1844)	Guangdong, Yunnan
	Filopaludina leei (Hsü, 1935)*	Guangxi
	Filopaludina sumatrensis (Dunker, 1852)	Yunnan
Idiopoma Pilsbry, 1901	*Idiopoma dissimilis* (O. F. Müller, 1774)	Yunan, Hainan
	Idiopoma umbilicata (I. Lea, 1856)	Hainan
Margarya Nevill, 1877*	*Margarya francheti* (Mabille, 1886)*	Yunnan
	Margarya dianchiensis (Zhang, 1990)*	Yunnan
	Margarya melanioides Nevill, 1877*	Yunnan
	Margarya monodi Dautzenberg & Fischer, 1906*	Yunnan
Mekongia Crosse & Fischer, 1876	*Mekongia liuiana* (Yen, 1937)*	Hunan
	Mekongia smithi (Yen, 1942)*	Hunan
Rivularia Heude, 1890*	*Rivularia auriculata* (E. von Martens, 1875)*	Hunan, Hubei, Jiangxi, Zhejiang, Fujian, Guangdong
	Rivularia bicarinata (Kobelt, 1909)*	Hunan
	Rivularia globosa Heude, 1890*	Hubei, Hunan, Guizhou
	Rivularia ovum Heude, 1890*	Hunan, Guizhou, Guangxi
Sinotaia Haas, 1939	*Sinotaia quadrata* (W. H. Benson, 1842)	All provinces except for Tibet
Tchangmargarya He, 2013*	*Tchangmargarya multilabiata* Zhang & Chen, 2015*	Yunnan
	Tchangmargarya yangtsunghaiensis(Tchang & Tsi, 1949)*	Yunnan
	Tchangmargarya ziyi Zhang, 2017*	Yunnan
Ussuripaludina Zatravkin & Bogatov, 1987	*Ussuripaludina ussuriensis* (Gerstfeldt, 1859)	Jilin, Heilongjiang
Viviparus Montfort, 1810	*Viviparus* cf. *viviparus* (Linnaeus, 1758)	Xinjiang

*endemic to China.

4.2 Systematics and origin of Truncatelloidea

The superfamily Truncatelloidea has the highest number of species among freshwater molluscs. Using detailed anatomic studies and molecular phylogenetics, the systematics below family/genus level of many truncatelloidean groups have been studied relatively well, such as in Hydrobiidae (e.g. Wilke, 2003; Szarowska *et al*., 2005; Boulaassafer *et al*., 2018; Delicado *et al*., 2019), Bythinellidae (e.g. Bichain *et al*., 2007; Benke *et al*., 2009; Wilke *et al*., 2010; Falniowski *et al*., 2012) and Moitessieriidae (e.g. Hofman *et al*., 2018; Falniowski *et al*., 2019; Grego *et al*., 2020; Rysiewska *et al*., 2021). However, systematic studies of Truncatelloidea above family level are quite scarce and without sufficient taxonomic coverage (e.g., Layton *et al*., 2019). Here, I present the most complete and reliable multi-locus phylogeny of Truncatelloidea above family level with one fossil calibration. The result confirmed again the recently suggested placement of *Helicostoa* within the widespread

family Bithyniidae, instead of the monotypic family Helicostoidae endemic to China (in chapter 5; Wilke *et al*., 2023). In addition, I proposed a new monospecific freshwater truncatelloidean family Squamapicidae, which is the first and only valid molluscan family endemic to China (in chapter 6).

The present phylogeny revealed relatively reliable family-level relationships within Truncatelloidea, especially the freshwater groups for the first time (in chapter 6). The phylogeny revealed two main clades of Truncatelloidea, the marine-dominant clade A with 10 families and the freshwater-dominant clade B (Hydrobioid) with 21 families, similar to the results of all former studies (e.g. Criscione & Ponder, 2013; Takano & Kano, 2014; Layton *et al*., 2019). The basal groups of the clade B are the families with true marine members (Cochliopidae, Tateidae, Hydrobiidae) or the families exclusive to marine or brackish water (Anabathridae, Hydrococcidae), indicating a marine origin of the clade B. The other 16 families of the clade B formed a highly supported monophyletic clade origin from the Early Cretaceous. Within this clade only one fourth (4/16) of families have true marine members (Assimineidae, Falsicingulidae, Truncatellidae, Stenothyridae), and the other families are all exclusively found in freshwater or brackish water (with much lower diversity). This monophyletic clade is divided into three highly supported sub-clades. The three sub-clades diverged into 16 extant families during the Late Cretaceous. A detailed discussion of the relationship between families is presented in chapter 6.

The oldest reliable fossil of Truncatelloidea is a freshwater species found in the Chinle Formation of North America. During the Late Triassic, the Chinle Formation was a continental environment with river, lakes and wetland located near the southwest coast of Laurasia (Kirby, 1993; Pott, 2012). Layton *et al*., (2019) revealed that Truncatelloidea is monophyletic, and the sister group of Truncatelloidea is a clade only including true marine gastropods (Eulimidae, Vanikoridae, Rissonidae, Zebinidae, Rissoidae, Emblandidae). It is therefore highly liklely that the ancestor of Truncatelloidea was marine and existed earlier than the freshwater species from the Late Triassic.

The basal lineages in the phylogeny shown herein may be able to provide more clues about the origin of Truncatelloidea. Spirostyliferinidae and Calopiidae form two relatively basal branches in clade A: Spirostyliferinidae is only known from Java Island to NW Australia (Layton *et al*., 2019), and Calopiidae is restrict to the northern to eastern coast of Australia (Ponder, 1999). The subclade of Hydrococcidae and Cochliopidae is the basal lineage of clade B: Hydrococcidae is restricted to the western to southern coast of Australia (Ponder, 1982), and Cochliopidae is mostly distributed in the Neotropics and Nearctic, with the highest diversity in Central America (Hershler and Thompson, 1992). Another relatively basal lineage of clade B is Tateidae, which is widely distributed in Australia, New Zealand, the Pacific islands and South America (Ponder in Lydeard & Cummings, 2019). It is notable that many basal lineages of Truncatelloidea are restricted to Australia or mainly distributed in Australia. During the Late Triassic, Australia was located in southeastern Gondwana, and the current western coast was facing the Tethys Ocean (McKellar, 2004). Chapter 6 revealed that the families

Squmapicidae and Stenothyridae probably originated from the Tethys Ocean. Meanwhile, the Mediterranean region with the highest diversity of hydrobioids is the largest relic of the Tethys Ocean. Based on the fossils and present phylogeny, there is a relatively high possibility that Truncatelloidea originated from the coastal waters of the southwest Tethys Ocean during the Triassic, and the marine ancestor of Truncatelloidea successfully entered the continental freshwater environment of Pangaea by the end of the Late Triassic.

In conclusion, based on the new material from China and a newly integrated mitochondrial and nuclear dataset of former published data, the present thesis fills the former gap of a robust time-calibrated phylogeny of Truncatelloidea, which is the key to understand the evolution and origination of this extremely diverse group. However, the sampling in the present phylogeny of several families, such as Hydrobiidae, Truncatellidae and Moitessieriidae, is still quite limited due to the lack of sequences of many groups beyond COI and 16S. A broader sequencing of nuclear genes or even genomic studies should help us to reconstructed a more robust and complete phylogeny in the future.

4.3 Evolution of Viviparidae and Truncatelloidea from China

Viviparidae and Truncatelloidea are the two most diverse groups of freshwater gastropods distributed in China. The large number of endemic species and genera, even an endemic family, not only underline the high biodiversity of freshwater gastropods in China, but also offer many good opportunities to study some basic and old, but nevertheless disputed questions of evolution, such as speciation or evolutionary innovation. Here, some broad evolutionary scenarios for Viviparidae and Truncatelloidea are discussed summarily.

4.3.1 Non-adaptive radiation of Viviparidae in insular habitats

Insular habitats, such as islands, lakes (“land islands”) or even mountains (“sky islands”), provide natural laboratories for the study of speciation. Many studies on radiations in insular habitats, such as those of the finches of the Galapagos Islands (Sato *et al*. 2001), the land snails of the Hawaii Islands (Cowie, 1995), the cichlids of the African Great Lakes (Meyer *et al*., 1994) and the freshwater gastropods of ancient lakes on Sulawesi Island (von Rintelen *et al*., 2004), have contributed significantly to our understanding of speciation, including adaptive and non-adaptive speciation.

Non-adaptive radiation refers to the diversification of a lineage without obvious ecological diversification, which is common in allopatric or parapatric taxa (Rundell & Price, 2009). In the present thesis, two cases of non-adaptive radiation in insular habitats have been discovered within Viviparidae, viz. *Dalipaludina* with four species endemic to the group of plateau lakes in Northwest Yunnan (chapter 2), and three endemic species of *Bakyietaia* from Hainan Island (chapter 4). All these viviaprid species have strictly allopatric distributions and similar ecological niches.

Dalipaludina is a genus mostly endemic to the plateau lakes in Northwest Yunnan, with an exception in Northeast Yunnan. These lakes are very close to each other, about 10 to 100 kilometres apart, and distributed along the northwestern border of the central Yunnan subregion of the Yangtze stratigraphic region (Zhang *et al*., 2015). However, each lake only harbours one *Dalipaludina* species, and each species is endemic to a single or several nearby lakes (details in chapter 2). Such an allopatric distribution pattern with narrow isolation also could be found in cases from oceanic islands, such as Galapagos tortoises (Caccone *et al*., 1999), Pacific monitor lizards (Weijola *et al*., 2019) and white-eye birds of the Solomon Islands (Manthey *et al*., 2020). However, this pattern is relatively rare in lakes. The viviparid genus *Anularya* endemic to the plateau lakes in Yunnan also displays such a strict allopatric distribution pattern ("one lake – one species") with narrow isolation: Fuxian Lake with *Anularya bicostata* (Tchang & Tsi, 1949) and Xingyun Lake with *Anularya mansuyi* (Dautzenberg & Fischer, 1906) are only 1.5 kilometers apart and connected by a river (Zhang *et al*., 2015). A similar case in cyprinid fishes is also found in these two lakes: *Anabarilius grahami* (Regan, 1908) endemic to Fuxian Lake and *Anabarilius andersoni* Regan, 1904 endemic to Xingyun Lake (Yang, 1992). The radiation of *Dalipaludina* in the plateau lakes in Northwest Yunnan provides a new and interesting case of non-adaptive speciation.

Bakyietaia is the most diverse genus of Viviparidae in China, with 15 species known from the country. Three species endemic to Hainan Island and one species endemic to Leizhou Peninsula cluster in a highly supported monophyletic clade. In chapter 4, I have discussed the origin of the endemic Hainan group from the Leizhou Peninsula, and the radiation within Hainan Island due to geographic separation. Notably, on relatively small Hainan Island a surprisingly high species diversity and uniqueness of *Bakyietaia* is found (7 species, 4 endemic), compared to the much larger continental regions like Guangdong (6 species, 2 endemic), Guangxi (9 species, 3 endemic), and Vietnam & Laos (4 species, 1 endemic). There is obviously the possibility of insufficient sampling of the continental regions. However, species radiations are indeed more common in insular environments as mentioned above. The house mice introduced to the Madeira Island by humans display an extremely rapid chromosomal radiation in less than 500 years, which is caused by the geographic isolation and genetic drift (Britton-Davidian, 2000). It is believed that morphological evolution is accelerated on islands compared to continents for mammals (Millien, 2006). There is no reason not to expect this phenomenon to happen in other taxa as well, such as land snail or freshwater gastropods in this case.

4.3.2 Evolutionary innovations in Truncatelloidea

Truncatelloidea displays an extremely wide adaption to different habitats. Within the entire animal kingdom, there is almost no other superfamily as Truncatelloidea that can be found from polar to tropical regions, from cold underground water to hot springs (*Stenothyra thermaecola* Kuroda, 1962, only found in several hot springs around 40 °C), from deep sea to high mountains. *Angustassiminea* Habe, 1943 and *Cavernacmella* Habe, 1942 of truncatelloidean family Assimineidae even have

marine, freshwater and terrestrial species in a single genus (Fukuda in Lydeard & Cummings, 2019). In addition, the fossil species *Triasamnicola pilsbryi* Yen & Reeside, 1946 from the Late Triassic, which represents the first known case of conquering freshwater habitats within gastropods, belongs to Truncatelloidea as well. These adaptions of Truncatelloidea definitely require enormous morphological and genetic evolutionary innovations.

In the present thesis, several remarkable evolutionary innovations of truncatelloidean snails have been studied systematically for the first time: the sessility, secondary aperture and extreme sexual dimorphism of genus *Helicostoa* (chapter 5), and the two-stage highly ornamented protoconch of the new family Squamapicidae (chapter 6). These evolutionary innovations have never been reported from any other freshwater gastropod (obligatory sessility, two-stage highly ornamented protoconch) or even entire Mollusca (extreme sexual dimorphism, secondary aperture).

Sessility is not overly rare in marine bivalves and gastropods, and is also reported from some freshwater bivalves (Sarà, 1986; Ricciardi, 1988; Bogan & Bouchet, 1998). However, within freshwater gastropods, species of *Helicostoa* and *Melanoides agglutinans* (Bequaert & Clench, 1941) are the only two known cases of sessility, and the sessile habit of *Melanoides agglutinans* is probably facultative (Bequaert & Clench, 1941). In the present thesis, the rediscovery of the living *Helicostoa* species from China provides important ecological information about its habitat for the first time: big rivers with a high throughflow and a lot of limestone inside. These limestones offer a special ecological niche for *Helicostoa*, and the innovation of sessility allows *Helicostoa* species to become the dominant freshwater gastropod on rocks where it occurs.

However, sessility creates an obvious obstacle for mating. A marine sessile gastropod species *Vermicularia spirata* (Philippi, 1836) is a protandrous hermaphrodite (Bieler & Hadfield, 1990). The free-living small males of *Vermicularia spirata* can search for and mate with sessile large females, which were males before completing sex reversal. Within Truncatelloidea, protandrous hermaphroditism is only reported from one marine species of the family Vitrinellidae (Bieler & Mikkelsen, 1988). We consider that *Helicostoa liuae* has evolved an extreme sexual dimorphism instead of protandrous hermaphroditism to overcome the obstacles for mating in sessile organisms: only females are sessile, and males are free-living; sessile females cement the original aperture on the rock, and develop a special opening on the body whorl as a secondary aperture for feeding, excretion and mating; free-living males have a normal shell and aperture, and can search for and copulate with sessile females. The gender of *Helicostoa* can even be identified based on the juvenile shell, and males can hardly undergo sex reversal into females due to the large differences of the adult shell. These evolutionary innovations seems to be further adaptions to a sessile life history, and have never reported from any other mollusc.

The protoconch displays the early stage development of the gastropod shell. Most freshwater gastropods only have a relatively simple and smooth protoconch. However, the freshwater gastropod

Squamapex taiji endemic to Fuxian Lake has a unique protoconch with two developmental stages, and a complicated microstructure differing from the surface structure of the adult whorls. As discussed in chapter 6, such a protoconch has never been reported from freshwater gastropods before, but is relatively common in marine or migratory gastropods with veliger larvae. This kind of protoconch or veliger larvae have never reported from the sister group of Squampicidae, viz. Stenothyridae, or any other related truncatelloidean gastropods, even though there are some marine species within these families. As a large (212 km^2), deep (155 m) and ancient (3.4 Myr old) lake, Fuxian Lake provides a unique habitat for this minute (1 mm in height) freshwater gastropod species. There are essentially two options to explain the two-stage protoconch in *Squamapex*: this species has either developed a veliger larval stage as an independent evolutionary innovation in this freshwater lake as it might convey similar benefits as in a marine environment, or, alternatively, this is an ancestral character retained after the marine-freshwater transition within the ancestral lineage. However, these hypotheses can only be tested based on detailed ecological, developmental and possibly also transcriptomic or genomic studies of living animals in the future.

Overall, the examples given here indicate the potential of Viviparidae and Truncatelloidea from China to serve as models for tackling long-standing questions in evolutionary biology. Undoubtedly, more detailed investigations will reveal even more of such interesting cases that deserve further comprehensive study combining ecological and genomic studies within a robust phylogenetic/phylogeneomic framework in the future.

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Appendix – Declaration of contributions to each publication and manuscript

Chapter 1. Zhang LJ & von Rintelen T (2021). The neglected operculum: a revision of the opercular characters in river snails (Caenogastropoda: Viviparidae). *Journal of Molluscan Studies* **87**: eyab008.

I designed the project and experiment, collected the materials for the study, did the measurements, took photos, performed most of the analyses, drafted and wrote the manuscript.

Chapter 2. Zhang LJ, Du LN & von Rintelen T (2023). A new genus of river snails, *Dalipaludina* (Gastropoda, Viviparidae), endemic to the Yunnan Plateau of SW China. *Zoosystematics and Evolution* **99**: 285-297

I designed the project and experiment, conducted the field work and collected most of the material for the study, did the measurements, did the experiment in the molecular and SEM lab, took photos, performed most of the morphological and molecular analyses, drafted and wrote the manuscript.

Chapter 3. Zhang LJ & Wang XD (2023). An extinct species of *Tchangmargarya* (Gastropoda: Viviparidae) from Quaternary lacustrine deposits of a group of vanished lakes in Yunnan, China. *Molluscan Research*, accepted

I designed the project and experiment, conducted the field work and collected parts of the material for the study, did the measurements, took photos, performed most of the morphological analysis, drafted and wrote the manuscript.

Chapter 4. Zhang LJ, Yen YH, Chen ZY, Du LN, Ng TH & von Rintelen T. A new genus of river snails, *Bakyietaia* (Mollusca, Viviparidae), from South China and mainland Southeast Asia. Manuscript.

I designed the project and experiment, conducted the field works and collected parts of the material for the study, took the measurements, did the experiments in the molecular and SEM lab, took photos, performed most of the morphological and molecular analyses, drafted and the manuscript.

Chapter 5. Zhang LJ, Shi ZA, Chen ZY, von Rintelen T, Zhang W & Lou ZJ. Rediscovery and systematics of the enigmatic genus *Helicostoa* reveals a new species of sessile freshwater snail with remarkable sexual dimorphism. Manuscript.

I designed the project and experiment, did the measurements, did parts of the experiment in the molecular and SEM lab, took photos, preformed most of morphology and molecular analysis, drafted and wrote the manuscript.

Chapter 6. Zhang LJ, Bernardes SC, Meng K & von Rintelen T. A new monospecific family of freshwater snail from China with Cretaceous Tethys origin. Manuscript.

I designed the project and experiment, conducted the field works and collected the material for the study, collected the published data for molecular analysis, did the measurements, did the experiment in the molecular and SEM lab, took photos, performed most of the morphological analysis and parts of the molecular analysis, drafted and wrote the manuscript.

I hereby confirm the above statement

Lejia Zhang Thomas von Rintelen